NOVEL PURINE INHIBITORS OF FRUCTOSE-1,6-BISPHOSPHATASE

Field of the Invention

This invention relates to novel purine compounds that are inhibitors of Fructose-1,6-bisphosphatase at the AMP site. The invention also relates to the preparation and

5   use of these purine analogs in the treatment of diabetes, and other diseases where the inhibition of gluconeogenesis, control of blood glucose levels, reduction in glycogen stores, or reduction in insulin levels is beneficial.

Background and Introduction to the Invention

Diabetes mellitus (or diabetes) is one of the most prevalent diseases in the world

10 today. Diabetes patients have been divided into two classes, namely type I or insulin-dependent diabetes mellitus and type II or non-insulin dependent diabetes mellitus (NIDDM). Non-insulin-dependent diabetes mellitus (NIDDM) accounts for approximately 90% of all diabetics and is estimated to affect 12-14 million adults in the U. S. alone (6.6% of the population). NIDDM is characterized by both fasting

15 hyperglycemia and exaggerated postprandial increases in plasma glucose levels. NIDDM is associated with a variety of long-term complications, including microvascular diseases such as retinopathy, nephropathy and neuropathy, and macrovascular diseases such as coronary heart disease. Numerous studies in animal models demonstrate a causal relationship between long term complications and

20 hyperglycemia. Recent results from the Diabetes Control and Complications Trial (DCCT) and the Stockholm Prospective Study demonstrate this relationship for the first time in man by showing that insulin-dependent diabetics with tighter glycemic control are at substantially lower risk for development and progression of these complications. Tighter control is also expected to benefit NIDDM patients.

25   Current therapies used to treat NIDDM patients entail both controlling lifestyle risk factors and pharmaceutical intervention. First-line therapy for NIDDM is typically

a tightly-controlled regimen of diet and exercise since an overwhelming number of

NIDDM patients are overweight or obese (~67%) and since weight loss can improve

insulin secretion, insulin sensitivity and lead to normoglycemia. Normalization of

blood glucose occurs in less than 30% of these patients due to poor compliance and

5   poor response. Patients with hyperglycemia not controlled by diet alone are

subsequently treated with oral hypoglycemics or insulin. Until recently, the

sulfonylureas were the only class of oral hypoglycemic agents available for NIDDM.

Treatment with sulfonylureas leads to effective blood glucose lowering in only 70% of

patients and only 40% after 10 years of therapy. Patients that fail to respond to diet and

10   sulfonylureas are subsequently treated with daily insulin injections to gain adequate

glycemic control.

Although the sulfonylureas represent a major therapy for NIDDM patients, four

factors limit their overall success. First, as mentioned above, a large segment of the

NIDDM population do not respond adequately to sulfonylurea therapy (*i.e.* primary

15   failures) or become resistant (*i.e.* secondary failures). This is particularly true in

NIDDM patients with advanced NIDDM since these patients have severely impaired

insulin secretion. Second, sulfonylurea therapy is associated with an increased risk of

severe hypoglycemic episodes. Third, chronic hyperinsulinemia has been associated

with increased cardiovascular disease although this relationship is considered

20   controversial and unproven. Last, sulfonylureas are associated with weight gain, which

leads to worsening of peripheral insulin sensitivity and thereby can accelerate the

progression of the disease.

Recent results from the U.K. Diabetes prospective study also showed that

patients undergoing maximal therapy of a sulfonylurea, metformin, or a combination of

25   the two, were unable to maintain normal fasting glycemia over the six year period of the

study. U.K. Prospective Diabetes Study 16. Diabetes, 44:1249-158 (1995). These

results further illustrate the great need for alternative therapies. Three therapeutic

strategies that could provide additional health benefits to NIDDM patients beyond the

currently available therapies, include drugs that would: (i) prevent the onset of NIDDM; (ii) prevent diabetic complications by blocking detrimental events precipitated by chronic hyperglycemia; or (iii) normalize glucose levels or at least decrease glucose levels below the threshold reported for microvascular and macrovascular diseases.

5   Hyperglycemia in NIDDM is associated with two biochemical abnormalities, namely insulin resistance and impaired insulin secretion. The relative roles of these metabolic abnormalities in the pathogenesis of NIDDM has been the subject of numerous studies over the past several decades. Studies of offspring and siblings of NIDDM patients, mono- and dizygotic twins, and ethnic populations with high

10   incidence of NIDDM (e.g. Pima Indians) strongly support the inheritable nature of the disease.

          Despite the presence of insulin resistance and impaired insulin secretion, fasting blood glucose (FBG) levels remain normal in pre-diabetic patients due to a state of compensatory hyperinsulinemia. Eventually, however, insulin secretion is inadequate

15   and fasting hyperglycemia ensues. With time insulin levels decline. Progression of the disease is characterized by increasing FBG levels and declining insulin levels.

          Numerous clinical studies have attempted to define the primary defect that accounts for the progressive increase in FBG. Results from these studies indicate that excessive hepatic glucose output (HGO) is the primary reason for the elevation in FBG

20   with a significant correlation found for HGO and FBG once FBG exceeds 140 mg/dL. Kolterman, et al., J. Clin. Invest. 68:957, (1981); DeFronzo Diabetes 37:667 (1988).

          HGO comprises glucose derived from breakdown of hepatic glycogen (glycogenolysis) and glucose synthesized from 3-carbon precursors (gluconeogenesis). A number of radioisotope studies and several studies using ^{13}C-NMR spectroscopy

25   have shown that gluconeogenesis contributes between 50-100% of the glucose produced by the liver in the postabsorptive state and that gluconeogenesis flux is excessive (2- to 3-fold) in NIDDM patients. Magnusson, et al. J. Clin. Invest. 90:1323-1327 (1992);

Rothman, et al., Science 254: 573-76 (1991); Consoli, et a l. Diabetes 38:550-557
(1989).

Gluconeogenesis from pyruvate is a highly regulated biosynthetic pathway
requiring eleven enzymes (Figure 1). Seven enzymes catalyze reversible reactions and
5   are common to both gluconeogenesis and glycolysis. Four enzymes catalyze reactions
unique to gluconeogenesis, namely pyruvate carboxylase, phosphoenolpyruvate
carboxykinase, fructose-1,6-bisphosphatase and glucose-6-phosphatase. Overall flux
through the pathway is controlled by the specific activities of these enzymes, the
enzymes that catalyzed the corresponding steps in the glycolytic direction, and by
10   substrate availability. Dietary factors (glucose, fat) and hormones (insulin, glucagon,
glucocorticoids, epinephrine) coordinatively regulate enzyme activities in the
gluconeogenesis and glycolysis pathways through gene expression and post-
translational mechanisms.

.Of the four enzymes specific to gluconeogenesis, fructose-1,6-bisphosphatase
15   (hereinafter "FBPase") is the most suitable target for a gluconeogenesis inhibitor based
on efficacy and safety considerations. Studies indicate that nature uses the FBPase/PFK
cycle as a major control point (metabolic switch) responsible for determining whether
metabolic flux proceeds in the direction of glycolysis or gluconeogenesis. Claus, et al.,
Mechanisms of Insulin Action, Belfrage, P. editor, pp.305-321, Elsevier Science 1992;
20   Regen, et al. J. Theor. Biol., 111:635-658 (1984); Pilkis, et al. Annu. Rev. Biochem,
57:755-783 (1988). FBPase is inhibited by fructose-2,6-bisphosphate in the cell.
Fructose-2,6-bisphosphate binds to the substrate site of the enzyme. AMP binds to an
allosteric site on the enzyme.

Synthetic inhibitors of FBPase have also been reported. McNiel reported that
25   fructose-2,6-bisphosphate analogs inhibit FBPase by binding to the substrate site. J.
Med. Chem., 106:7851 (1984); U.S. Patent No. 4,968,790 (1984). These compounds,
however, were relatively weak and did not inhibit glucose production in hepatocytes
presumably due to poor cell penetration.

Gruber reported that some nucleosides can lower blood glucose in the whole animal through inhibition of FBPase. These compounds exert their activity by first undergoing phosphorylation to the corresponding monophosphate. EP 0 427 799 B1.

Gruber et al. U.S. Patent No. 5,658,889 described the use of inhibitors of the

5   AMP site of FBPase to treat diabetes.

European patent application EP 0 632 048 A1 discloses certain ethyl phosphonates of purine derivatives for use as antiviral and antineoplastic agents. These structures differ from the claimed compounds because they have no substitution on the C-8 of the purine. There is no suggestion that these compounds are inhibitors of

10   FBPase.

Brief Description of the Drawings

FIG. 1 is a scheme depicting the eleven enzymes of the gluconeogenesis pathway.

FIG. 2 shows the dose-dependent inhibition of hPFBPase AMP and compounds

15   2.7, 2.13, and 2.5.

FIG. 3 shows the displacement of ^{3}H-AMP from hPFBPase by ZMP and compound 2.2.

FIG. 4A depicts the reduction of glucose production from dihydroxyacetone in rat hepatocytes treated with compound 2.7.

20   FIG. 4B depicts the increase in the amount of fructose-1,6-bisphosphate in rat hepatocytes exposed to dihydroxyacetone and treated with compound 2.7.

FIG. 5 depicts a dose-dependent inhibition of glucose production in rat hepatocytes exposed to lactate and pyruvate by compounds 2.7 and 2.1.

FIG. 6 shows the effect of various doses of compound 16.4 on blood glucose

25   levels in 18 hour fasted, normal rats.

FIG. 7 is a bar graph depicting the reduction in blood glucose levels in 18 hour fasted normal rats treated with compound 2.7 given at a dose of 20 mg/kg i.p.

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FIG. 8 is a bar graph depicting the increased accumulation of fructose-1,6-bisphosphate in the liver of 18-hour fasted rats treated with compound 2.7.

FIG. 9A is a bar graph depicting a reduction in blood glucose levels in 24 hour fasted Zucker Diabetic Fatty rats treated with compound 2.7.

5   FIG. 9B is a bar graph depicting the percentage change in blood glucose levels in 24 hour fasted Zucker Diabetic Fatty rats treated with compound 2.7.

FIG. 10 depicts the inhibition of gluconeogenesis from ^{14}C-bicarbonate in 24 hour fasted Diabetic Fatty rats treated with compound 2.7.

FIG. 11A depicts the dose-dependent inhibition of glucose production in rat 10   hepatocytes by compound 16.4, a prodrug of compound 2.7.

FIG. 11B shows the intracellular generation of compound 2.7 in rat hepatocytes treated with compound 16.4, a prodrug, to inhibit glucose production in rat hepatocytes.
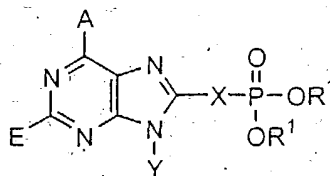
Summary of the Invention

The present invention is directed towards novel purine compounds which bind 15   the AMP site and are potent FBPase inhibitors. In another aspect, the present invention is directed to the preparation of these novel purine compounds and to the *in vitro* and *in vivo* FBPase inhibitory activity of these compounds. Another aspect of the present invention is directed to the clinical use of the novel FBPase inhibitors as a method of treatment or prevention of diseases responsive to inhibition of gluconeogenesis and in 20   diseases responsive to lowered blood glucose levels.

Gruber et al. U.S. patent application Serial Number 08/355,836 (allowed) described the use of inhibitors of the AMP site of FBPase to treat diabetes.

The compounds are also useful in treating or preventing excess glycogen storage diseases and insulin dependent diseases such as cardiovascular diseases including 25   atherosclerosis.

The invention comprises the novel purine analogs as specified below in formula 1. Also included in the scope of the present invention are prodrugs of the compounds of formula 1.

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$$\text{(structure: purine ring with substituent A at top, E at left, Y on N at bottom, and side chain } -X-\overset{\displaystyle O}{\underset{\displaystyle OR^1}{\overset{\|}{P}}}-OR^1 \text{)}$$

Formula 1

Since these compounds may have asymmetric centers, the present invention is directed not only to racemic mixtures of these compounds, but also to individual

5   stereoisomers. The present invention also includes pharmaceutically acceptable and/or useful salts of the compounds of formula 1, including acid addition salts and basic salts. The present inventions also encompass prodrugs of compounds of formula 1.

Definitions

In accordance with the present invention and as used herein, the following terms

10 are defined with the following meanings, unless explicitly stated otherwise.

The term "aryl" refers to aromatic groups which have at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted.

Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring

15 are carbon atoms. Carbocyclic aryl groups include monocyclic carbocyclic aryl groups and polycyclic or fused compounds such as optionally substituted naphthyl groups.

Heterocyclic aryl groups are groups having from 1 to 4 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms being carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen. Suitable heteroaryl groups

20 include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolyl, pyridyl-N-oxide, pyrimidyl, pyrazinyl, imidazolyl, and the like, all optionally substituted.

The term "biaryl" represents aryl groups containing more than one aromatic ring including both fused ring systems and aryl groups substituted with other aryl groups.

The term "alicyclic" means compounds which combine the properties of aliphatic and cyclic compounds and include but are not limited to aromatic, cycloalkyl and bridged cycloalkyl compounds. The cyclic compound includes heterocycles. Cyclohexenylethyl, cyclohexanylethyl, and norbornyl are suitable alicyclic groups.

5   Such groups may be optionally substituted.

The term "optionally substituted" or "substituted" includes groups substituted by one to four substituents, independently selected from lower alkyl, lower aryl, lower aralkyl, lower alicyclic, hydroxy, lower alkoxy, lower aryloxy, perhaloalkoxy, aralkoxy, heteroaryl, heteroaryloxy, heteroarylalkyl, heteroaralkoxy, azido, amino,

10 guanidino, halogen, lower alkylthio, oxa, ketone, carboxy esters, carboxyl, carboxamido, nitro, acyloxy, alkylamino, aminoalkyl, alkylaminoaryl, alkylaryl, alkylaminoalkyl, alkoxyaryl, arylamino, aralkylamino, phosphonate, sulfonate, carboxamidoalkylaryl, carboxamidoaryl, hydroxyalkyl, haloalkyl, alkylaminoalkylcarboxy, aminocarboxamidoalkyl, cyano, lower alkoxyalkyl, and lower

15 perhaloalkyl.

The term "aralkyl" refers to an alkyl group substituted with an aryl group. Suitable aralkyl groups include benzyl, picolyl, and the like, and may be optionally substituted.

The term "lower" referred to herein in connection with organic radicals or

20 compounds respectively defines such as with up to and including 10, preferably up to and including 6, and advantageously one to four carbon atoms. Such groups may be straight chain, branched, or cyclic.

The terms "arylamino" (a), and "aralkylamino" (b), respectively, refer to the group -NRR' wherein respectively, (a) R is aryl and R' is hydrogen, alkyl, aralkyl or

25 aryl, and (b) R is aralkyl and R' is hydrogen or aralkyl, aryl, alkyl.

The term "acyl" refers to -C(O)R where R is alkyl and aryl.

The term "carboxy esters" refers to -C(O)OR where R is alkyl, aryl, aralkyl, and alicyclic, all optionally substituted.

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The term "oxa" refers to $=O$ in an alkyl group.

The term "alkylamino" refers to -NRR' where R and R' are independently selected from hydrogen or alkyl.

The term "carbonylamine" or "carbonylamino" refers to -CONR$_2$ where each R is independently hydrogen or alkyl.

The term "halogen" or "halo" refers to -F, -Cl, -Br and -I.

The term "oxyalkylamino" refers to -O-alk-NR-, where "alk" is an alkylene group and R is H or alkyl.

The term "alkylsulfonate" refers to the group -alk-S(O)$_2$-O- where "alk" is an alkylene group.

The term "alkylaminoalkylcarboxy" refers to the group -alk-NR-alk-C(O)-O- where "alk" is an alkylene group, and R is a H or lower alkyl.

The term "alkylaminocarbonyl" refers to the group -alk-NR-C(O)- where "alk" is an alkylene group, and R is a H or lower alkyl.

The term "oxyalkyl" refers to the group -O-alk- where "alk" is an alkylene group.

The term "alkylcarboxyalkyl" refers to the group -alk-C(O)-O-alkyl where each alk is independently an alkylene group.

The term "alkyl" refers to saturated aliphatic groups including straight-chain, branched chain and cyclic groups. Alkyl groups may be optionally substituted.

The term "bidentate" refers to an alkyl group that is attached by its terminal ends to the same atom to form a cyclic group. For example, propylene imine contains a bidentate propylene group.

The term "cyclic alkyl" refers to alkyl groups that are cyclic.

The term "heterocyclic" and "heterocyclic alkyl" refer to cyclic alkyl groups containing at least one heteroatom. Suitable heteroatoms include oxygen, sulfur, and nitrogen. Heterocyclic groups may be attached through a heteroatom or through a carbon atom in the ring.

The term "alkenyl" refers to unsaturated groups which contain at least one carbon-carbon double bond and includes straight-chain, branched-chain and cyclic groups. Alkene groups may be optionally substituted.

The term "alkynyl" refers to unsaturated groups which contain at least one
5 carbon-carbon triple bond and includes straight-chain, branched-chain and cyclic groups. Alkyne groups may be optionally substituted.

The term "alkylene" refers to a divalent straight chain, branched chain or cyclic saturated aliphatic radical.

The term "acyloxy" refers to the ester group -O-C(O)R, where R is H, alkyl,
10 alkenyl, alkynyl, aryl, aralkyl, or alicyclic.

The term "alkylaryl" refers to the group -alk-aryl- where "alk" is an alkylene group. "Lower alkylaryl" refers to such groups where alkylene is lower alkyl.

The term "alkylamino" refers to the group -alk-NR- wherein "alk" is an alkylene group.

15 The term "alkyl(carboxyl)" refers to carboxyl substituted off the alkyl chain. Similarly, "alkyl(hydroxy)", "alkyl(phosphonate)", and "alkyl(sulfonate)" refers to substituents off the alkyl chain.

The term "alkylaminoalkyl" refers to the group -alk-NR-alk- wherein each "alk" is an independently selected alkylene, and R is H or lower alkyl. "Lower
20 alkylaminoalkyl" refers to groups where each alkylene group is lower alkyl.

The term "alkylaminoaryl" refers to the group -alk-NR-aryl- wherein "alk" is an alkylene group. In "lower alkylaminoaryl", the alkylene group is lower alkyl.

The term "alkyloxyaryl" refers to an alkylene group substituted with an aryloxy group. In "lower alkyloxyaryl", the alkylene group is lower alkyl.

25 The term "alkylacylamino" refers to the group -alk-N-(COR)- wherein alk is alkylene and R is lower alkyl. In "lower alkylacylamino", the alkylene group is lower alkyl.

The term "alkoxyalkylaryl" refers to the group -alk-O-alk-aryl- wherein each "alk" is independently an alkylene group. "Lower aloxyalkylaryl" refers to such groups where the alkylene group is lower alkyl.

The term "alkylacylaminoalkyl refers to the group -alk-N-(COR)-alk- where each alk is an independently selected alkylene group. In "lower alkylacylaminoalkyl" the alkylene groups are lower alkyl.

The term "alkoxy" refers to the group -alk-O- wherein alk is an alkylene group.

The term "alkoxyalkyl" refers to the group -alk-O-alk- wherein each alk is an independently selected alkylene group. In "lower alkoxyalkyl", each alkylene is lower alkyl.

The term "alkylthio" refers to the group -alk-S- wherein alk is alkylene group.

The term "alkylthioalkyl" refers to the group -alk-S-alk- wherein each alk is an independently selected alkylene group. In "lower alkylthioalkyl" each alkylene is lower alkylene.

The term "aralkylamino" refers to an amine substituted with an aralkyl group.

The term "alkylcarboxamido" refers to the group -alk- C(O)N(R)- wherein alk is an alkylene group and R is H or lower alkyl.

The term "alkylcarboxamidoalkyl" refers to the group -alk-C(O)N(R)-alk- wherein each alk is an independently selected alkylene group and R is lower alkyl. In "lower alkylcarboxamidoalkyl" each alkylene is lower alkyl.

The term "alkylcarboxamidoalkylaryl" refers to the group -alk_1-C(O)-NH-alk_2Ar- wherein alk_1 and alk_2 are independently selected alkylene groups and alk_2 is substituted with an aryl group, Ar. In "lower alkylcarboxamidoalkylaryl", each alkylene is lower alkyl.

The term "heteroalicyclic" refers to an alicyclic group having 1 to 4 heteroatoms selected from nitrogen, sulfur, phosphorus and oxygen.

The term "aminocarboxamidoalkyl" refers to the group -NH-C(O)-N(R)-R wherein each R is an independently selected alkyl group. "Lower aminocaboxamidoalkyl" refers to such groups wherein each R is lower alkyl.

The term "heteroarylalkyl" refers to an alkyl group substituted with a heteroaryl

5   group.

The term "perhalo" refers to groups wherein every C-H bond has been replaced with a C-halo bond on an aliphatic or aryl group. Suitable perhaloalkyl groups include -CF$_3$ and -CFCl$_2$.

The term "guanidine" refers to both -NR-C(NR)-NR$_2$ as well as -N=C(NR$_2$)$_2$

10   where each R group is independently selected from the group of -H, alkyl, alkenyl, alkynyl, aryl, and alicyclic, all optionally substituted.

The term "amidine" refers to -C(NR)-NR$_2$ where each R group is independently selected from the group of -H, alkyl, alkenyl, alkynyl, aryl, and alicyclic, all optionally substituted.

15   The term "pharmaceutically acceptable salt" includes salts of compounds of formula 1 and its prodrugs derived from the combination of a compound of this invention and an organic or inorganic acid or base.

The term "prodrug" as used herein refers to any compound that when administered to a biological system generates the "drug" substance either as a result of

20   spontaneous chemical reaction(s) or by enzyme catalyzed or metabolic reaction(s). Reference is made to various prodrugs such as acyl esters, carbonates, and carbamates, included herein. The groups illustrated are exemplary, not exhaustive, and one skilled in the art could prepare other known varieties of prodrugs. Such prodrugs of the compounds of formula 1, fall within the scope of the present invention.

25   The term "prodrug ester" as employed herein includes, but is not limited to, the following groups and combinations of these groups:

[1]   Acyloxyalkyl esters which are well described in the literature (Farquhar et al., J. Pharm. Sci. 72, 324-325 (1983)) and are represented by formula A
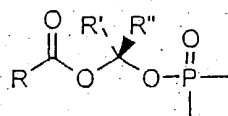
Formula A

wherein　　　R, R', and R" are independently H, alkyl, aryl, alkylaryl, and
alicyclic; (see WO 90/08155; WO 90/10636).

[2]　　Other acyloxyalkyl esters are possible in which an alicyclic ring is
formed such as shown in formula B. These esters have been shown to generate
phosphorus-containing nucleotides inside cells through a postulated sequence of
reactions beginning with deesterification and followed by a series of elimination
reactions (e.g. Freed et al., Biochem. Pharm. 38: 3193-3198 (1989)).
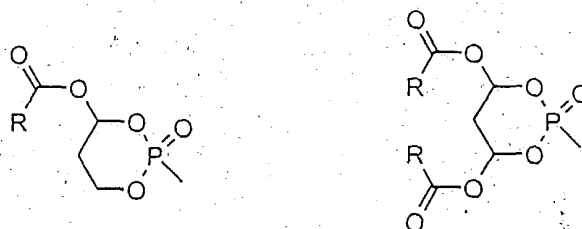
Formula B

wherein R is -H, alkyl, aryl, alkylaryl, alkoxy, aryloxy, alkylthio, arylthio,
alkylamino, arylamino, cycloalkyl, or alicyclic.

[3]　　Another class of these double esters known as
alkyloxycarbonyloxymethyl esters, as shown in formula A, where R is alkoxy, aryloxy,
alkylthio, arylthio, alkylamino, and arylamino; R', and R" are independently H, alkyl,
aryl, alkylaryl, and alicyclic, have been studied in the area of β-lactam antibiotics
(Tatsuo Nishimura et al. J. Antibiotics, 1987, 40(1), 81-90; for a review see Ferres, H.,

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Drugs of Today, 1983,*19*, 499.). More recently Cathy, M. S., et al. (Abstract from
AAPS Western Regional Meeting, April, 1997) showed that these
alkyloxycarbonyloxymethyl ester prodrugs on (9-[(R)-2-
phosphonomethoxy)propyl]adenine (PMPA) are bioavailable up to 30% in dogs.

5   [4]   Aryl esters have also been used as phosphonate prodrugs (e.g. Erion,
DeLambert et al., J. Med. Chem. 37: 498, 1994; Serafinowska et al., J. Med. Chem. 38:
1372, 1995). Phenyl as well as mono and poly-substituted phenyl phosphonate ester
prodrugs have generated the parent phosphonic acid in studies conducted in animals and
in man (Formula C). Another approach has been described where Y is a carboxylic
10   ester ortho to the phosphate. Khamnei and Torrence, J. Med. Chem.; 39:4109-4115
(1996).
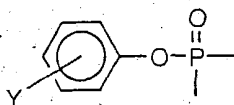
Formula C

wherein   Y is H, alkyl, aryl, alkylaryl, alkoxy, acetoxy, halogen, amino,
15   alkoxycarbonyl, hydroxy, cyano, alkylamino, and alicyclic.

         [5]   Benzyl esters have also been reported to generate the parent phosphonic
acid. In some cases, using substituents at the para-position can accelerate the
hydrolysis. Benzyl analogs with 4-acyloxy or 4-alkyloxy group [Formula D, X = H,
OR or O(CO)R or O(CO)OR] can generate the 4-hydroxy compound more readily
20   through the action of enzymes, e.g. oxidases, esterases, etc. Examples of this class of
prodrugs are described by Mitchell et al., J. Chem. Soc. Perkin Trans. I 2345 (1992);
Brook, et al. WO 91/19721.
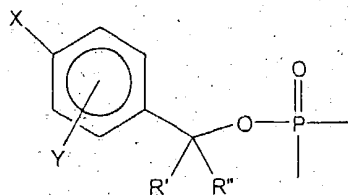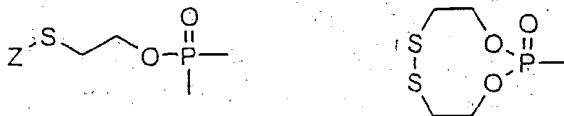
Formula D

wherein   X and Y are independently H, alkyl, aryl, alkylaryl, alkoxy, acetoxy,
               hydroxy, cyano, nitro, perhaloalkyl, halo, or alkyloxycarbonyl; and

5   R' and R" are independently H, alkyl, aryl, alkylaryl, halogen, and
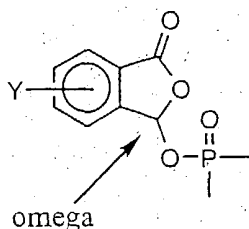   alicyclic.

       [6]   Thio-containing phosphonate phosphonate ester prodrugs have been
described that are useful in the delivery of FBPase inhibitors to hepatocytes. These
phosphonate ester prodrugs contain a protected thioethyl moiety as shown in formula E.
10   One or more of the oxygens of the phosphonate can be esterified. Since the mechanism
that results in de-esterification requires the generation of a free thiolate, a variety of
thiol protecting groups are possible. For example, the disulfide is reduced by a
reductase-mediated process (Puech et al., Antiviral Res., 22: 155-174 (1993)).
Thioesters will also generate free thiolates after esterase-mediated hydrolysis.
15   Benzaria, et al., J. Med. Chem., 39:4958 (1996). Cyclic analogs are also possible and
were shown to liberate phosphonate in isolated rat hepatocytes. The cyclic disulfide
shown below has not been previously described and is novel.

Formula E

wherein Z is alkylcarbonyl, alkoxycarbonyl, arylcarbonyl, aryloxycarbonyl, or alkylthio.
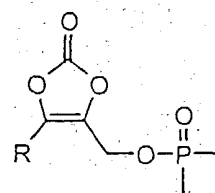
Other examples of suitable prodrugs include proester classes exemplified by Biller and Magnin (U.S. Patent No. 5,157,027); Serafinowska et al. (J. Med. Chem. 38, 1372 (1995)); Starrett et al. (J. Med. Chem. 37, 1857 (1994)); Martin et al. J. Pharm. Sci. 76, 180 (1987); Alexander et al., Collect. Czech. Chem. Commun, 59, 1853 (1994)); and EPO patent application 0 632 048 A1. Some of the structural classes described are optionally substituted, including fused lactones attached at the omega position and optionally substituted 2-oxo-1,3-dioxolenes attached through a methylene to the phosphorus oxygen such as:
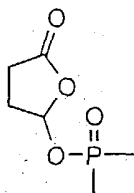
3-phthalidyl   2-oxotetrahydrofuran-5-yl   2-oxo-4,5-didehydro-1,3-
                                                                dioxolanemethyl

wherein R is -H, alkyl, cycloalkyl, or alicyclic; and

wherein Y is -H, alkyl, aryl, alkylaryl, cyano, alkoxy, acetoxy, halogen, amino, alkylamino, alicyclic, and alkoxycarbonyl.

[7]   Propyl phosphonate ester prodrugs can also be used to deliver FBPase inhibitors into hepatocytes. These phosphonate ester prodrugs may contain a hydroxyl and hydroxyl group derivatives at the 3-position of the propyl group as shown in formula F. The R and X groups can form a cyclic ring system as shown in formula F. One or more of the oxygens of the phosphonate can be esterified.
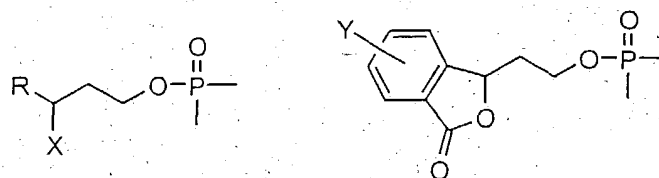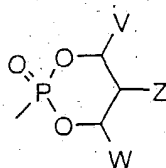
Formula F

wherein   R is alkyl, aryl, heteroaryl;

X is hydrogen, alkylcarbonyloxy, alkyloxycarbonyloxy; and

Y is alkyl, aryl, heteroaryl, alkoxy, alkylamino, alkylthio, halogen,

5   hydrogen, hydroxy, acetoxy, amino.

[8]   The cyclic propyl phosphonate esters as in Formula G are shown to activate to phosphonic acids. The activation of prodrug can be mechanistically explained by *in vivo* oxidation and elimination steps. These prodrugs inhibit glucose production in isolated rat hepatocytes and are also shown to deliver FBPase inhibitors to

10 the liver following oral administration.

Formula G

wherein

V and W are independently selected from the group consisting of hydrogen,

15 aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, 1-alkynyl, and -R^9; or

together V and Z are connected to form a cyclic group containing 3-5 atoms, optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarboxy, or aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached to the phosphorus; or

20   together V and W are connected to form a cyclic group containing 3 carbon atoms substituted with hydroxy, acyloxy, alkoxycarboxy, alkylthiocarboxy,

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hydroxymethyl, and aryloxycarboxy attached to a carbon atom that is three atoms from

an oxygen attached to the phosphorus;

Z is selected from the group consisting of -CH_2OH, -CH_2OCOR^3,

-CH_2OC(O)SR^3, -CH_2OCO_2R^3, -SR^3, -S(O)R^3, -CH_2N_3, -CH_2NR^2_2, -CH_2Ar,

5   -CH(Ar)OH, -CH(CH=CR^2R^2)OH, -CH(C=CR^2)OH, and -R^2;

with the provisos that:

a)   V, Z, W are not all -H; and

b)   when Z is -R^2, then at least one of V and W is not -H or -R^9;

R^2 is selected from the group consisting of R^3 and -H;

10   R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl; and

R^9 is selected from the group consisting of alkyl, aralkyl, and alicyclic.

[9]   Phosphoramidate derivatives have been explored as potential

phosphonate prodrugs (e.g. McGuigan et al., *Antiviral Res.* 1990, *14*: 345; 1991, *15 :*

255. Serafinowska et al., *J. Med. Chem.*, 1995, *38*, 1372). Most phosphoramidates are

15   unstable under aqueous acidic conditions and are hydrolyzed to the corresponding

phosphonic acids. Cyclic phosphoramidates have also been studied as phosphonate

prodrugs because of their potential for greater stability compared to non cyclic

phosphoramidates (e.g. Starrett et al., *J. Med. Chem.*, 1994, 37: 1857).

Other prodrugs are possible based on literature reports such as substituted ethyls

20   for example, bis(trichloroethyl)esters as disclosed by McGuigan, et al. Bioorg Med.

Chem. Lett., 3:1207-1210 (1993), and the phenyl and benzyl combined nucleotide

esters reported by Meier, C. et al. Bioorg. Med. Chem. Lett., 7:99-104 (1997).

X group nomenclature as used herein in formula 1 describes the group attached

to the phosphonate and ends with the group attached to the 2-position of the

25   benzimidazole ring. For example, when X is alkylamino, the following structure is

intended:
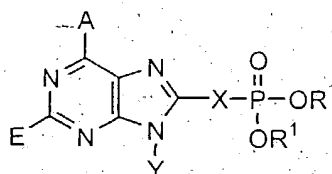
(purine ring)-NR-alk-O-P(O)(OR^1)_2
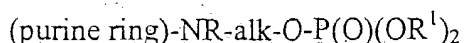
Y group nomenclature likewise ends with the group attached to the ring.

DETAILED DESCRIPTION OF THE INVENTION

Novel Purine Compounds

Preferred compounds of the present invention are inhibitors of the AMP site of
5   FBPase of the following formula (1):

wherein

A is selected from the group consisting of $-NR^8_2$, $NHSO_2R^3$, $-OR^5$, $-SR^5$,
halogen, lower alkyl, $-CON(R^4)_2$, guanidine, amidine, -H, and perhaloalkyl;

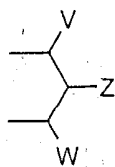10   E is selected from the group consisting of -H, halogen, lower alkylthio, lower
perhaloalkyl, lower alkyl, lower alkenyl, lower alkynyl, lower alkoxy, -CN, and $-NR^7_2$;

X is selected from the group consisting of alkylamino, alkyl, alkenyl, alkynyl,
alkyl(carboxyl), alkyl(hydroxy), alkyl(phosphonate), alkyl(sulfonate), aryl,
alkylaminoalkyl, alkoxyalkyl, alkylthioalkyl, alkylthio, alicyclic, 1,1-dihaloalkyl,
15   carbonylalkyl, aminocarbonylamino, alkylaminocarbonyl, alkylcarbonylamino, aralkyl,
and alkylaryl, all optionally substituted; or together with Y forms a cyclic group
including cyclic alkyl, heterocyclic, and aryl;

Y is selected from the group consisting of -H, alkyl, alkenyl, alkynyl, aryl,
alicyclic, aralkyl, aryloxyalkyl, alkoxyalkyl, $-C(O)R^3$, $-S(O)_2R^3$, $-C(O)-OR^3$, $-CONHR^3$,
20   $-NR^2_2$, and $-OR^3$, all except H are optionally substituted; or together with X forms a
cyclic group including aryl, cyclic alkyl, and heterocyclic;

R^1 is independently selected from the group consisting of -H, alkyl, aryl,
alicyclic where the cyclic moiety contains a carbonate or thiocarbonate, $-C(R^2)_2$-aryl,
alkylaryl, $-C(R^2)_2OC(O)NR^2_2$, $-NR^2-C(O)-R^3$, $-C(R^2)_2-OC(O)R^3$, $C(R^2)_2-O-C(O)OR^3$,

$-C(R^2)_2OC(O)SR^3$, alkyl-S-C(O)R^3, alkyl-S-S-alkylhydroxy, and alkyl-S-S-S-alkylhydroxy, or together R^1 and R^1 are -alkyl-S-S-alkyl to form a cyclic group, or together R^1 and R^1 are

5   wherein

V and W are independently selected from the group consisting of hydrogen, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, 1-alkynyl, and -R^9; or

together V and Z are connected to form a cyclic group containing 3-5 atoms, optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarboxy, or

10   aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached to the phosphorus; or

together V and W are connected to form a cyclic group containing 3 carbon atoms substituted with hydroxy, acyloxy, alkoxycarboxy, alkylthiocarboxy, hydroxymethyl, and aryloxycarboxy attached to a carbon atom that is three atoms from

15   an oxygen attached to the phosphorus;

Z is selected from the group consisting of -CH_2OH, -CH_2OCOR^3, -$CH_2OC(O)SR^3$, -$CH_2OCO_2R^3$, -SR^3, -$S(O)R^3$, -CH_2N_3, -$CH_2NR^2_2$, -CH_2Ar, -CH(Ar)OH, -CH(CH=CR^2R^2)OH, -CH(C≡CR^2)OH, and -R^2;

with the provisos that:

20   a)   V, Z, W are not all -H; and

b)   when Z is -R^2, then at least one of V and W is not -H or -R^9;

R^2 is selected from the group consisting of R^3 and -H;

R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl;

R^4 is independently selected from the group consisting of -H, lower alkyl, lower alicyclic, lower aralkyl, and lower aryl;

R^5 is selected from the group consisting of lower alkyl, lower aryl, lower aralkyl, and lower alicyclic;

R^6 is independently selected from the group consisting of -H, and lower alkyl;

R^7 is independently selected from the group consisting of -H, lower alkyl, lower alicyclic, lower aralkyl, lower aryl, and -C(O)R^{10};

R^8 is independently selected from the group consisting of -H, lower alkyl, lower aralkyl, lower aryl, lower alicyclic, -C(O)R^{10}, or together they form a bidendate alkyl;

R^9 is selected from the group consisting of alkyl, aralkyl, and alicyclic;

R^{10} is selected from the group consisting of -H, lower alkyl, -NH$_2$, lower aryl, and lower perhaloalkyl;

R^{11} is selected from the group consisting of alkyl, aryl, -OH, -NH$_2$ and -OR^3; and

pharmaceutically acceptable prodrugs and salts thereof.

Preferred Compounds of Formula 1

Suitable alkyl groups include groups having from 1 to about 20 carbon atoms. Suitable aryl groups include groups having from 1 to about 20 carbon atoms. Suitable aralkyl groups include groups having from 2 to about 21 carbon atoms. Suitable acyloxy groups include groups having from 1 to about 20 carbon atoms. Suitable alkylene groups include groups having from 1 to about 20 carbon atoms. Suitable alicyclic groups include groups having 3 to about 20 carbon atoms. Suitable heteroaryl groups include groups having from 1 to about 20 carbon atoms and from 1 to 5 heteroatoms, preferably independently selected from nitrogen, oxygen, phosphorous, and sulfur. Suitable heteroalicyclic groups include groups having from 2 to about twenty carbon atoms and from 1 to 5 heteroatoms, preferably independently selected from nitrogen, oxygen, phosphorous, and sulfur.

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Preferred A groups include $-NR^8_2$, lower alkyl, lower perhaloalkyl, lower alkoxy, and halogen. Particularly preferred are $-NR^8_2$, and halogen. Especially preferred is $-NR^8_2$. Most preferred is $-NH_2$.

Preferred E groups include -H, halogen, lower perhaloalkyl, -CN, lower alkyl,

5   lower alkoxy, and lower alkylthio. Particularly preferred E groups include -H, -SMe, -Et, and -Cl. Especially preferred is -H and $-SCH_3$.

Preferred X groups include alkylamino, alkyl, alkynyl, alkoxyalkyl, alkylthio, aryl, 1,1-dihaloalkyl, carbonylalkyl, heteroaryl, alkylcarbonylamino, and alkylaminocarbonyl. Particularly preferred is alkyl substituted with 1 to 3 substituents

10   selected from halogen, phosphonate, $-CO_2H$, $-SO_3H$, and -OH. Particularly preferred are alkylaminocarbonyl, alkoxyalkyl, and heteroaryl. Preferred alkoxyalkyl groups include methoxymethyl. Preferred heteroaryl groups include furanyl, optionally substituted.

Preferred Y groups include aralkyl, alicyclic, alkyl, and aryl, all optionally

15   substituted. Particularly preferred is lower alkyl. Particularly preferred Y groups include (2-naphthyl)methyl, cyclohexylethyl, phenylethyl, nonyl, cyclohexylpropyl, ethyl, cyclopropylmethyl, cyclobutylmethylphenyl, (2-methyl)propyl, neopentyl, cyclopropyl, cyclopentyl, (1-imidozolyl)propyl, 2-ethoxybenzyl, 1-hydroxy-2,2-dimethylpropyl, 1-chloro-2,2-dimethylpropyl, 2,2-dimethylbutyl , 2-(spiro-3',3'-

20   dimethylcyclohex-4-enyl)propyl, and 1-methylneopentyl. Especially preferred is neopentyl and isobutyl.

Preferred R^4 and R^7 groups are -H, and lower alkyl. Particularly preferred are -H, and methyl.

Preferred R^1 groups include -H, alkyl, aryl, alicyclic where the cyclic moiety

25   contains a carbonate or thiocarbonate, $-C(R^2)_2$-aryl, alkylaryl, $-C(R^2)_2OC(O)NR^2_2$, $-NR^2-C(O)-R^3$, $-C(R^2)_2-OC(O)R^3$, $C(R^2)_2-O-C(O)OR^3$, $-C(R^2)_2OC(O)SR^3$, alkyl-S-C(O)R^3, alkyl-S-S-alkylhydroxy, and alkyl-S-S-S-alkylhydroxy, or together R^1 and R^1 are -alkyl-S-S-alkyl to form a cyclic group, or together R^1 and R^1 are

$$\begin{array}{c} V \\ \diagup \\ \diagdown \text{-Z} \\ \diagup \\ W \end{array},$$
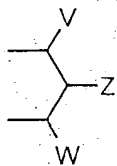
wherein

V and W are independently selected from the group consisting of hydrogen, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, 1-alkynyl, and $-R^9$; or

5   together V and Z are connected to form a cyclic group containing 3-5 atoms, optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarboxy, or aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached to the phosphorus; or

together V and W are connected to form a cyclic group containing 3 carbon

10   atoms substituted with hydroxy, acyloxy, alkoxycarboxy, alkylthiocarboxy, hydroxymethyl, and aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached to the phosphorus;

Z is selected from the group consisting of $-CH_2OH$, $-CH_2OCOR^3$, $-CH_2OC(O)SR^3$, $-CH_2OCO_2R^3$, $-SR^3$, $-S(O)R^3$, $-CH_2N_3$, $-CH_2NR^2{}_2$, $-CH_2Ar$,

15   $-CH(Ar)OH$, $-CH(CH=CR^2R^2)OH$, $-CH(C\equiv CR^2)OH$, and $-R^2$;

with the provisos that:

a)   V, Z, W are not all -H; and

b)   when Z is $-R^2$, then at least one of V and W is not -H or $-R^9$;

R^2 is selected from the group consisting of R^3 and -H;

20   R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl; and

R^9 is selected from the group consisting of alkyl, aralkyl, and alicyclic.

Preferred R^1 groups include -H, alkylaryl, aryl, $-C(R^2)_2$-aryl, and $-C(R^2)_2$ $-OC(O)R^3$. Preferred such R^1 groups include optionally substituted phenyl, optionally substituted benzyl, -H, $-C(R^2)_2OC(O)OR^3$, and $-C(R^2)_2OC(O)R^3$. Preferably, said alkyl

25   groups are greater than 4 carbon atoms. Another preferred aspect is where at least one R^1 is aryl or $-C(R^2)_2$-aryl. Also particularly preferred are compounds where R^1 is

23

alicyclic where the cyclic moiety contains carbonate or thiocarbonate. Another

preferred aspect is when at least one R^1 is $-C(R^2)_2-OC(O)R^3$, $-C(R^2)_2-OC(O)OR^3$ or -

$C(R^2)_2-OC(O)SR^3$. Also particularly preferred is when R^1 and R^1 together are

optionally substituted, including fused, lactone attached at the omega position or are

5   optionally substituted 2-oxo-1,3-dioxolenes attached through a methylene to the

phosphorus oxygen. Also preferred is when at least one R^1 is -alkyl-S-S-alkylhydroxyl,

-alkyl-S-C(O)R^3, and -alkyl-S-S-S-alkylhydroxy, or together R^1 and R^1 are -alkyl-S-S-

alkyl- to form a cyclic group. Also preferred is where R^1 and R^1 together are

10   to form a cyclic group,

wherein

V and W are independently selected from the group consisting of hydrogen,

aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, 1-alkynyl, and -R^9; or

together V and Z are connected to form a cyclic group containing 3-5 atoms,

15   optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarboxy, or

aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached

to the phosphorus; or

together V and W are connected to form a cyclic group containing 3 carbon

atoms substituted with hydroxy, acyloxy, alkoxycarboxy, alkylthiocarboxy,

20   hydroxymethyl, and aryloxycarboxy attached to a carbon atom that is three atoms from

an oxygen attached to the phosphorus;

Z is selected from the group consisting of -CH_2OH, -CH_2OCOR^3,

-$CH_2OC(O)SR^3$, -$CH_2OCO_2R^3$, -SR^3, -$S(O)R^3$, -CH_2N_3, -$CH_2NR^2_2$, -CH_2Ar,

-$CH(Ar)OH$, -$CH(CH=CR^2R^2)OH$, -$CH(C\equiv CR^2)OH$, and -R^2;

25   with the provisos that:

24

a)   V, Z, W are not all -H; and

b)   when Z is -R^2, then at least one of V and W is not -H or -R^9;
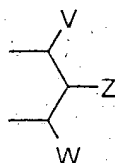
R^2 is selected from the group consisting of R^3 and -H;

R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl; and

R^9 is selected from the group consisting of alkyl, aralkyl, and alicyclic.

Particularly preferred are such groups wherein V and W both form a 6-membered carbocyclic ring substituted with 0-4 groups, selected from the group consisting of hydroxy, acyloxy, alkoxycarbonyl, and alkoxy; and Z is R^2. Also particularly preferred are such groups wherein V and W are hydrogen; and Z is selected from the group consisting of hydroxyalkyl, acyloxyalkyl, alkyloxyalkyl, and alkoxycarboxyalkyl. Also particularly preferred are such groups wherein V and W are independently selected from the group consisting of hydrogen, optionally substituted aryl, and optionally substituted heteroaryl, with the proviso that at least one of V and W is optionally substituted aryl or optionally substituted heteroaryl.

In one preferred aspect, R^1 is not lower alkyl of 1-4 carbon atoms.

In another preferred aspect, A is -NR^8_2 or halogen, E is -H, halogen, -CN, lower alkyl, lower perhaloalkyl, lower alkoxy, or lower alkylthio, X is alkylamino, alkyl, alkoxyalkyl, alkynyl, 1,1-dihaloalkyl, carbonylakyl, alkyl(OH), alkyl(sulfonate), alkylcarbonylamino, alkylaminocarbonyl, alkylthio, aryl, or heteroaryl, and R^4 and R^7 is -H or lower alkyl. Particularly preferred are such compounds where Y is aralkyl, aryl, alicyclic, or alkyl. Especially preferred are such compounds where R^1 and R^1 together are

wherein

V and W are independently selected from the group consisting of hydrogen, aryl, substituted aryl, heteroaryl, substituted heteroaryl, 1-alkenyl, 1-alkynyl, and -R^9; or

25

together V and Z are connected to form a cyclic group containing 3-5 atoms,

optionally 1 heteroatom, substituted with hydroxy, acyloxy, alkoxycarboxy, or

aryloxycarboxy attached to a carbon atom that is three atoms from an oxygen attached

to the phosphorus; or

5   together V and W are connected to form a cyclic group containing 3 carbon

atoms substituted with hydroxy, acyloxy, alkoxycarboxy, alkylthiocarboxy,

hydroxymethyl, and aryloxycarboxy attached to a carbon atom that is three atoms from

an oxygen attached to the phosphorus;

Z is selected from the group consisting of $-CH_2OH$, $-CH_2OCOR^3$,

10 $-CH_2OC(O)SR^3$, $-CH_2OCO_2R^3$, $-SR^3$, $-S(O)R^3$, $-CH_2N_3$, $-CH_2NR^2_2$, $-CH_2Ar$,

$-CH(Ar)OH$, $-CH(CH=CR^2R^2)OH$, $-CH(C\equiv CR^2)OH$, and $-R^2$;

with the provisos that:

a)   V, Z, W are not all -H; and

b)   when Z is $-R^2$, then at least one of V and W is not -H or $-R^9$;

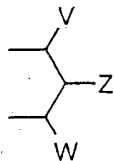15 R^2 is selected from the group consisting of R^3 and -H;

R^3 is selected from the group consisting of alkyl, aryl, alicyclic, and aralkyl; and

R^9 is selected from the group consisting of alkyl, aralkyl, and alicyclic.

In another preferred aspect, A is $-NR^8_2$, E is -H, Cl-, or methylthio, and X is

optionally substituted furanyl, or alkoxyalkyl. Particularly preferred are such

20 compounds where A is $-NH_2$, X is 2,5-furanyl, or methoxymethyl, and Y is lower alkyl.

Most preferred are such compounds where E is H, X is 2,5-furanyl, and Y is neopentyl;

those where E is $-SCH_3$, X is 2,5-furanyl, and Y is isobutyl; and those where E is -H, X

is 2,5-furanyl, and Y is 1-(3-chloro-2,2-dimethyl)-propyl. Especially preferred are such

compounds where R^1 is $-CH_2O-C(O)-C(CH_3)_3$.

25   In the following examples of preferred compounds, the following prodrugs are

preferred:

Acyloxyalkyl esters;

Alkoxycarbonyloxyalkyl esters;

26

Aryl esters;

Benzyl and substituted benzyl esters;

Disulfide containing esters;

Substituted (1,3-dioxolen-2-one)methyl esters;

5   Substituted 3-phthalidyl esters;

Cyclic-[2'-hydroxymethyl]-1,3-propanyl diesters and hydroxy protected forms;

Lactone type esters; and all mixed esters resulted from possible combinations of above

esters.

Bis-pivaloyloxymethyl esters;

10   Bis-isobutyryloxymethyl esters;

Cyclic-[2'-hydroxymethyl]-1,3-propanyl diester;

Cyclic-[2'-acetoxymethyl]-1,3-propanyl diester;

Cyclic-[2'-methyloxycarbonyloxymethyl]-1,3-propanyl diester;

Bis-benzoylthiomethyl esters;

15   Bis-benzoylthioethyl esters;

Bis-benzoyloxymethyl esters;

Bis-p-fluorobenzoyloxymethyl esters;

Bis-6-chloronicotinoyloxymethyl esters;

Bis-5-bromonicotinoyloxymethyl esters;

20   Bis-thiophenecarbonyloxymethyl esters;

Bis-2-furoyloxymethyl esters;

Bis-3-furoyloxymethyl esters;

Diphenyl esters;

Bis-(4-methoxyphenyl) esters;

25   Bis-(2-methoxyphenyl) esters;

Bis-(2-ethoxyphenyl) esters;

Mono-(2-ethoxyphenyl) esters;

Bis-(4-acetamidophenyl) esters;

Bis-(4-aceyloxyphenyl) esters;

Bis-(4-hydroxyphenyl) esters;

Bis-(2-acetoxyphenyl) esters;

Bis-(3-acetoxyphenyl) esters;

5   Bis-(4-morpholinophenyl) esters;

Bis-[4-(1-triazolophenyl) esters;

Bis-(3-*N*,*N*-dimethylaminophenyl) esters;

Bis-(2-tetrahydronapthyl) esters;

Bis-(3-chloro-4-methoxy)benzyl esters;

10   Bis-(3-bromo-4-methoxy)benzyl esters;

Bis-(3-cyano-4-methoxy)benzyl esters;

Bis-(3-chloro-4-acetoxy)benzyl esters;

Bis-(3-bromo-4-acetoxy)benzyl esters;

Bis-(3-cyano-4-acetoxy)benzyl esters;

15   Bis-(4-chloro)benzyl esters;

Bis-(4-acetoxy)benzyl esters;

Bis-(3,5-dimethoxy-4-acetoxy)benzyl esters;

Bis-(3-methyl-4-acetoxy)benzyl esters;

Bis-(benzyl)esters;

20   Bis-(3-methoxy-4-acetoxy)benzyl esters;

Bis-(3-chloro-4-acetoxy)benzyl esters;

cyclic-(2,2-dimethylpropyl)phosphonoamidate;

cyclic-(2-hydroxymethylpropyl) ester;

Bis-(6'-hydroxy-3',4'-disulfide)hexyl esters;

25   Bis-(6'-acetoxy-3',4'-disulfide)hexyl esters;

(3',4'-*Dithia*)cyclononane esters;

Bis-(5-methyl-1,3-dioxolen-2-one-4-yl)methyl esters;

Bis-(5-ethyl-1,3-dioxolen-2-one-4-yl)methyl esters;

Bis-(5-tert-butyl-1,3-dioxolen-2-one-4-yl)methyl esters;

Bis-3-(5,6,7-trimethoxy)phthalidyl esters;

Bis-(cyclohexyloxycarbonyloxymethyl) esters;

Bis-(isopropyloxycarbonyloxymethyl) esters;

5   Bis-(ethyloxycarbonyloxymethyl) esters;

Bis-(methyloxycarbonyloxymethyl) esters;

Bis-(isopropylthiocarbonyloxymethyl) esters;

Bis-(phenyloxycarbonyloxymethyl) esters;

Bis-(benzyloxycarbonyloxymethyl) esters;

10   Bis-(phenylthiocarbonyloxymethyl) esters;

Bis-(p-methoxyphenyloxycarbonyloxymethyl) esters;

Bis-(m-methoxyphenyloxycarbonyloxymethyl) esters;

Bis-(o-methoxyphenyloxycarbonyloxymethyl) esters;

Bis-(o-methylphenyloxycarbonyloxymethyl) esters;

15   Bis-(p-chlorophenyloxycarbonyloxymethyl) esters;

Bis-(1,4-biphenyloxycarbonyloxymethyl) esters;

Bis-[(2-phthalimidoethyl)oxycarbonyloxymethyl]esters;

Bis-(N-Phenyl, N-methylcarbamoyloxymethyl) esters;

Bis-(2-trichloroethyl) esters;

20   Bis-(2-bromoethyl) esters;

Bis-(2-iodoethyl) esters;

Bis-(2-azidoethyl) esters;

Bis-(2-acetoxyethyl) esters;

Bis-(2-aminoethyl) esters;

25   Bis-(2-N,N-diaminoethyl) esters;

Bis-(2-aminoethyl) esters;

Bis-(methoxycarbonylmethyl) esters;

Bis-(2-aminoethyl) esters;

Bis-[*N,N*-di(2-hydroxyethyl)]amidomethylesters;

Bis-(2-aminoethyl) esters;

Bis-(2-methyl-5-thiozolomethyl) esters;

Bis-(bis-2-hydroxyethylamidomthyl) esters.

5   Most preferred are the following:

Bis-pivaloyloxymethyl esters;

Bis-isobutyryloxymethyl esters;

cyclic-(2-hydroxymethylpropyl) ester;

cyclic-(2-acetoxymethylpropyl) ester;

10 .   cyclic-(2-methyloxycarbonyloxymethylpropyl) ester;

cyclic-(2-cyclohexylcarbonyloxymethylpropyl)ester;

cyclic-(2-aminomethylpropyl)ester;

cyclic-(2-azidomethylpropyl)ester;

Bis-benzoylthiomethyl esters;

15   Bis-benzoylthioethylesters;

Bis-benzoyloxymethyl esters;

Bis-*p*-fluorobenzoyloxymethyl esters;

Bis-6-chloronicotinoyloxymethyl esters;

Bis-5-bromonicotinoyloxymethyl esters;

20   Bis-thiophenecarbonyloxymethyl esters;

Bis-2-furoyloxymethyl esters;

Bis-3-furoyloxymethyl esters;

Diphenyl esters;

Bis-(2-methyl)phenyl esters;

25   Bis-(2-methoxy)phenyl esters;

Bis-(2-ethoxy)phenyl esters;

Bis-(4-methoxy)phenyl esters;

Bis-(3-bromo-4-methoxy)benzyl esters;

Bis-(4-acetoxy)benzyl esters;

Bis-(3,5-dimethoxy-4-acetoxy)benzyl esters;

Bis-(3-methyl-4-acetoxy)benzyl esters;

Bis-(3-methoxy-4-acetoxy)benzyl esters;

5   Bis-(3-chloro-4-acetoxy)benzyl esters;

Bis-(cyclohexyloxycarbonyloxymethyl) esters;

Bis-(isopropyloxycarbonyloxymethyl) esters;

Bis-(ethyloxycarbonyloxymethyl) esters;

Bis-(methyloxycarbonyloxymethyl) esters;

10   Bis-(isopropylthiocarbonyloxymethyl) esters;

Bis-(phenyloxycarbonyloxymethyl) esters;

Bis-(benzyloxycarbonyloxymethyl) esters;

Bis-(phenylthiocarbonyloxymethyl) esters;

Bis-(*p*-methoxyphenyloxycarbonyloxymethyl) esters;

15   Bis-(*m*-methoxyphenyloxycarbonyloxymethyl) esters;

Bis-(o-methoxyphenyloxycarbonyloxymethyl) esters;

Bis-(o-methylphenyloxycarbonyloxymethyl) esters;

Bis-(*p*-chlorophenyloxycarbonyloxymethyl) esters;

Bis-(1,4-biphenyloxycarbonyloxymethyl) esters;

20   Bis-[(2-phthalimidoethyl)oxycarbonyloxymethyl]esters;

Bis-(6'-hydroxy-3',4'-disulfide)hexyl esters; and

(3',4'-Disulfide)cyclononane esters.

Bis-(2-bromoethyl) esters;

Bis-(2-aminoethyl) esters;

25   Bis-(2-*N*,*N*-diaminoethyl) esters;

        Examples of preferred compounds include, but are not limited to those described
in Table 1 including salts and prodrugs thereof:

31
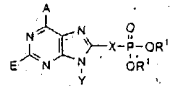
Table Compound No.	Synthetic Example No.				
		A	E	Y	X
1	2.7	NH2	H	neopentyl	2,5-furanyl
2		H	H	neopentyl	2,5-furanyl
3		Me	H	neopentyl	2,5-furanyl
4		Et	H	neopentyl	2,5-furanyl
5		Pr	H	neopentyl	2,5-furanyl
6		I	H	neopentyl	2,5-furanyl
7		Br	H	neopentyl	2,5-furanyl
8		Cl	H	neopentyl	2,5-furanyl
9		F	H	neopentyl	2,5-furanyl
10		OMe	H	neopentyl	2,5-furanyl
11		OEt	H	neopentyl	2,5-furanyl
12		OPr	H	neopentyl	2,5-furanyl
13		SMe	H	neopentyl	2,5-furanyl
14		SEt	H	neopentyl	2,5-furanyl
15		SPr	H	neopentyl	2,5-furanyl
16		SBn	H	neopentyl	2,5-furanyl
17		OBn	H	neopentyl	2,5-furanyl
18		NHMe	H	neopentyl	2,5-furanyl
19		NHEt	H	neopentyl	2,5-furanyl
20		NH-cPr	H	neopentyl	2,5-furanyl
21		NHOH	H	neopentyl	2,5-furanyl
22		NHNH2	H	neopentyl	2,5-furanyl
23		NHCHO	H	neopentyl	2,5-furanyl
24		NHAc	H	neopentyl	2,5-furanyl
25		NHCOCF3	H	neopentyl	2,5-furanyl
26		NHSO2Me	H	neopentyl	2,5-furanyl
27		CONH2	H	neopentyl	2,5-furanyl
28		ONH2	H	neopentyl	2,5-furanyl
29	22.2	NH2	SMe	neopentyl	2,5-furanyl
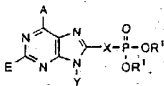
Table Compound No.	Synthetic Example No.			![structure]	
30		H	SMe	neopentyl	2,5-furanyl
31		Me	SMe	neopentyl	2,5-furanyl
32		Et	SMe	neopentyl	2,5-furanyl
33		Pr	SMe	neopentyl	2,5-furanyl
34		I	SMe	neopentyl	2,5-furanyl
35		Br	SMe	neopentyl	2,5-furanyl
36		Cl	SMe	neopentyl	2,5-furanyl
37		F	SMe	neopentyl	2,5-furanyl
38		OMe	SMe	neopentyl	2,5-furanyl
39		OEt	SMe	neopentyl	2,5-furanyl
40		OPr	SMe	neopentyl	2,5-furanyl
41		SMe	SMe	neopentyl	2,5-furanyl
42		SEt	SMe	neopentyl	2,5-furanyl
43		SPr	SMe	neopentyl	2,5-furanyl
44		SBn	SMe	neopentyl	2,5-furanyl
45		OBn	SMe	neopentyl	2,5-furanyl
46		NHMe	SMe	neopentyl	2,5-furanyl
47		NHEt	SMe	neopentyl	2,5-furanyl
48		NH-cPr	SMe	neopentyl	2,5-furanyl
49		NHOH	SMe	neopentyl	2,5-furanyl
50		NHNH2	SMe	neopentyl	2,5-furanyl
51		NHCHO	SMe	neopentyl	2,5-furanyl
52		NHAc	SMe	neopentyl	2,5-furanyl
53		NHCOCF3	SMe	neopentyl	2,5-furanyl
54		NHSO2Me	SMe	neopentyl	2,5-furanyl
55		CONH2	SMe	neopentyl	2,5-furanyl
56		ONH2	SMe	neopentyl	2,5-furanyl
57		NH2	H	isobutyl	2,5-furanyl
58	2.6	NH2	H	isopropyl	2,5-furanyl
59	2.5	NH2	H	ethyl	2,5-furanyl
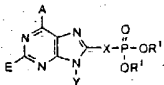
Table Compound No.	Synthetic Example No.			![structure] A N X-P-OR' E N Y OR' O	
60		NH2	H	methyl	2,5-furanyl
61	2.9	NH2	H	cyclopropyl	2,5-furanyl
62		NH2	H	cyclobutyl	2,5-furanyl
63	2.10	NH2	H	cyclopentyl	2,5-furanyl
64		NH2	H	cyclohexyl	2,5-furanyl
65		NH2	H	cycloheptanyl	2,5-furanyl
66		NH2	H	cyclopropylmethyl	2,5-furanyl
67		NH2	H	cyclobutylmethyl	2,5-furanyl
68		NH2	H	cyclopentylmethyl	2,5-furanyl
69		NH2	H	2-cyclopropylethyl	2,5-furanyl
70		NH2	H	2-cyclobutylethyl	2,5-furanyl
71		NH2	H	2-cyclopentylethyl	2,5-furanyl
72	2.2	NH2	H	2-cyclohexylethyl	2,5-furanyl
73	2.1	NH2	H	2-phenylethyl	2,5-furanyl
74		NH2	H	benzyl	2,5-furanyl
75		NH2	H	phenyl	2,5-furanyl
76		NH2	H	D-ribosyl	2,5-furanyl
77		NH2	H	H	2,5-furanyl
78		NH2	H	1-naphthylmethyl	2,5-furanyl
79	2.3	NH2	H	2-naphthylmethyl	2,5-furanyl
80		NH2	H	3-cyclopropylpropyl	2,5-furanyl
81		NH2	H	3-cyclobutylpropyl	2,5-furanyl
82		NH2	H	3-cyclopentylpropyl	2,5-furanyl
83		NH2	H	3-cyclohexylpropyl	2,5-furanyl
84	2.4	NH2	H	2,2-diphenylethyl	2,5-furanyl
85	2.8	NH2	H	adamentylmethyl	2,5-furanyl
86	2.11	NH2	H	2-ethoxybenzyl	2,5-furanyl
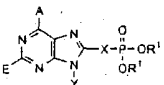
Table Compound No.	Synthetic Example No.				
87	2.13	NH2	H	2,2-dimethyl-3-hydroxy-1-propyl	2,5-furanyl
88	2.12	NH2	H	2,2-dimethyl-3-dimethylamino-1-propyl	2,5-furanyl
89	2.14	NH2	H	2,2-dimethyl-3-chloro-1-propyl	2,5-furanyl
90	2.15	NH2	H	3,3-dimethyl-1-butyl	2,5-furanyl
91	2.17	NH2	H	1,2,2-trimethyl-1-propyl	2,5-furanyl
92	2.16	NH2	H	1,5,5-trimethyl-3-ene-1-cyclohexylmethyl	2,5-furanyl
93		NH2	H	4-pyrimidylmethyl	2,5-furanyl
94		NH2	H	2-(4-pyrimidyl)ethyl	2,5-furanyl
95		NH2	H	5-pyrimidylmethyl	2,5-furanyl
96		NH2	H	2-(5-pyrimidyl)ethyl	2,5-furanyl
97		NH2	H	2-pyrimidylmethyl	2,5-furanyl
98		NH2	H	2-(2-pyrimidyl)ethyl	2,5-furanyl
99		NH2	H	2-pyridylmethyl	2,5-furanyl
100		NH2	H	2-(2-pyridyl)ethyl	2,5-furanyl
101		NH2	H	3-pyridylmethyl	2,5-furanyl
102		NH2	H	2-(3-pyridyl)ethyl	2,5-furanyl
103		NH2	H	4-pyridylmethyl	2,5-furanyl
104		NH2	H	2-(4-pyridyl)ethyl	2,5-furanyl
105		NH2	H	2-carbamoylethyl	2,5-furanyl
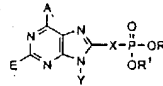
Table Compound No.	Synthetic Example No.				
106		NH2	H	1-(2-carbamoyl)propyl	2,5-furanyl
107		NH2	H	neopentyl	CONHCH2
108		NH2	H	neopentyl	CONHCH2CH2
109		NH2	H	neopentyl	CH2CH2CH2
110		NH2	H	neopentyl	CH2CH2CF2
111	5.5	NH2	H	neopentyl	CH2OCH2
112		NH2	H	neopentyl	CH2OCF2
113		NH2	H	neopentyl	CF2CF2CF2
114		NH2	H	neopentyl	acetylene
115		NH2	H	neopentyl	SCH2
116		NH2	H	neopentyl	SCH2CH2
117		NH2	H	neopentyl	CH2SCH2
118		NH2	H	neopentyl	NHCH2CH2
119		NH2	H	neopentyl	N(Ac)CH2CH2
120		NH2	H	neopentyl	N(Bz)CH2CH2
121		NH2	H	neopentyl	N(Me)CH2CH2
122		NH2	H	neopentyl	N(Bn)CH2CH2
123		NH2	H	neopentyl	NHCOCH2
124		NH2	H	neopentyl	NHCOCH2CH2
125		NH2	H	neopentyl	NHCOCF2
126		NH2	H	neopentyl	NHSO2CH2
127		NH2	H	neopentyl	N(Me)COCH2
128		NH2	H	neopentyl	N(Bn)COCH2
129		NH2	H	neopentyl	NHOCH2
130		NH2	H	neopentyl	CH2CH2CH(OH)
131		NH2	H	neopentyl	CH2CH2CH(CO2H)
132		NH2	H	neopentyl	CH2CH2CH(SO3H)
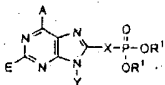
Table Compound No.	Synthetic Example No.			![structure]	
133		NH2	H	neopentyl	CH2CH2CH(PO3H2)
134	20.1	NH2	H	neopentyl	CH2-(1,2-imidazyl)
135		NH2	H	neopentyl	CH2-(1,2-pyrrolyl)
136		NH2	H	neopentyl	CSNHCH2
137		NH2	H	neopentyl	2,5-tetrahydrofuranyl
138		NH2	H	neopentyl	2,5-pyrrolidinyl
139		NH2	H	neopentyl	3,4-dihydroxy-2,5-tetrahydrofuranyl
140		NH2	H	neopentyl	2,4-furanyl
141		NH2	H	neopentyl	4,2-furanyl
142		NH2	H	neopentyl	2,5-thienyl
143		NH2	H	neopentyl	2,4-thienyl
144		NH2	H	neopentyl	4,2-thienyl
145		NH2	H	neopentyl	2,5-pyrrolyl
146		NH2	H	neopentyl	2,5-imidazyl
147		NH2	H	neopentyl	5,2-imidazyl
148		NH2	H	neopentyl	2,5-oxazyl
149		NH2	H	neopentyl	5,2-oxazyl
150	12.1	NH2	H	neopentyl	3,4-dichloro-2,5-furanyl
151		NH2	H	neopentyl	3-chloro-2,5-furanyl
152		NH2	H	neopentyl	4-chloro-2,5-furanyl
153		NH2	H	neopentyl	3,4-fluoro-2,5-
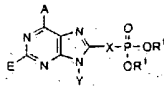
Table Compound No.	Synthetic Example No.				
					furanyl
154		NH2	H	neopentyl	3-fluoro-2,5-furanyl
155		NH2	H	neopentyl	4-fluoro-2,5-furanyl
156		NH2	H	neopentyl	CONHCH(CO2H)
157		NH2	Me	neopentyl	2,5-furanyl
158		NH2	Et	neopentyl	2,5-furanyl
159		NH2	Pr	neopentyl	2,5-furanyl
160		NH2	vinyl	neopentyl	2,5-furanyl
161		NH2	acetylenyl	neopentyl	2,5-furanyl
162		NH2	allyl	neopentyl	2,5-furanyl
163		NH2	2-furanyl	neopentyl	2,5-furanyl
164		NH2	3-furanyl	neopentyl	2,5-furanyl
165		NH2	2-thienyl	neopentyl	2,5-furanyl
166		NH2	3-thienyl	neopentyl	2,5-furanyl
167		NH2	Ph	neopentyl	2,5-furanyl
168	22.1	NH2	NH2	neopentyl	2,5-furanyl
169		NH2	NHMe	neopentyl	2,5-furanyl
170		NH2	N(Me)2	neopentyl	2,5-furanyl
171		NH2	NHBn	neopentyl	2,5-furanyl
172		NH2	I	neopentyl	2,5-furanyl
173		NH2	Br	neopentyl	2,5-furanyl
174		NH2	Cl	neopentyl	2,5-furanyl
175		NH2	F	neopentyl	2,5-furanyl
176		NH2	OMe	neopentyl	2,5-furanyl
177		NH2	OEt	neopentyl	2,5-furanyl
178		NH2	OPr	neopentyl	2,5-furanyl
179		NH2	SO2Me	neopentyl	2,5-furanyl
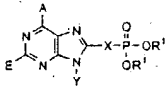
Table Compound No.	Synthetic Example No.				
180		NH2	SEt	neopentyl	2,5-furanyl
181		NH2	SPr	neopentyl	2,5-furanyl
182		NH2	SBu	neopentyl	2,5-furanyl
183		NH2	CN	neopentyl	2,5-furanyl
184		NH2	CONH2	neopentyl	2,5-furanyl
185		NH2	2-pyridyl	neopentyl	2,5-furanyl
186		NH2	3-pyridyl	neopentyl	2,5-furanyl
187		NH2	4-pyridyl	neopentyl	2,5-furanyl
188	5.4	NH2	H	1-(3-cyclohexyl)propyl	CH2OCH2
189	5.3	NH2	H	1-nonyl	CH2OCH2
190	5.2	NH2	H	2-cyclohexylethyl	CH2OCH2
191	5.1	NH2	H	2-phenethyl	CH2OCH2
192	10.2	NHMe	H	2-phenethyl	2,5-furanyl
193	10.1	N(Me)2	H	2-phenethyl	2,5-furanyl
194	9.1	Cl	H	2-phenethyl	2,5-furanyl
195	11.1	NH2	SMe	isobutyl	2,5-furanyl
196	11.2	NH2	SO2Me	isobutyl	2,5-furanyl
197	4.1	NH2	H	D-ribosyl	NHCH2CH2
198	4.2	NH2	H	5'-deoxy-D-ribosyl	NHCH2CH2
199		NH2	H	H	NHCH2CH2
200	3.1	NH2	H	benzyl	NHCH2CH2
201	3.2	NH2	H	2-phenethyl	NHCH2CH2
202	3.3	NH2	H	2-naphthylmethyl	NHCH2CH2
203	6.2	NH2	H	2-phenethyl	CH2CH2CH2
204	3.4	NH2	H	2-cyclohexylethyl	NHCH2CH2
205	6.1	NH2	H	2-cyclohexylethyl	CH2CH2CH2
206	8.1	NH2	H	2-cyclohexylethyl	SCH2
207	7.1	NH2	H	2-phenethyl	2,5-thienyl
208		NH2	Me	isobutyl	2,5-furanyl
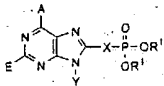
Table Compound No.	Synthetic Example No.			![structure]	
209		NH2	Et	isobutyl	2,5-furanyl
210		NH2	SEt	isobutyl	2,5-furanyl
211		NH2	SPr	isobutyl	2,5-furanyl
212		NH2	2-furanyl	isobutyl	2,5-furanyl
213		NH2	2-thienyl	isobutyl	2,5-furanyl
214		NH2	Pr	isobutyl	2,5-furanyl
215		NH2	F	isobutyl	2,5-furanyl
216		NH2	Cl	isobutyl	2,5-furanyl
217		NH2	Br	isobutyl	2,5-furanyl
218		NH2	H	isobutyl	2,5-furanyl
219		NH2	Et	isobutyl	CONHCH2
220		NH2	SEt	isobutyl	CONHCH2
221		NH2	SPr	isobutyl	CONHCH2
222		NH2	2-furanyl	isobutyl	CONHCH2
223		NH2	2-thienyl	isobutyl	CONHCH2
224		NH2	Pr	isobutyl	CONHCH2
225		NH2	F	isobutyl	CONHCH2
226		NH2	Cl	isobutyl	CONHCH2
227		NH2	Br	isobutyl	CONHCH2
228		NH2	Me	isobutyl	CONHCH2
229		NH2	H	isobutyl	CONHCH2
230		NH2	Et	neopentyl	acetylene
231		NH2	SEt	neopentyl	acetylene
232		NH2	SPr	neopentyl	acetylene
233		NH2	2-furanyl	neopentyl	acetylene
234		NH2	2-thienyl	neopentyl	acetylene
235		NH2	Pr	neopentyl	acetylene
236		NH2	F	neopentyl	acetylene
237		NH2	Cl	neopentyl	acetylene
238		NH2	Br	neopentyl	acetylene

40
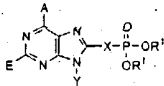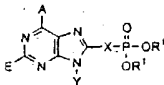
Table Compound No.	Synthetic Example No.				
239		NH2	Me	neopentyl	acetylene
240		NH2	Et	neopentyl	NHCOCH2
241		NH2	SEt	neopentyl	NHCOCH2
242		NH2	SPr	neopentyl	NHCOCH2
243		NH2	2-furanyl	neopentyl	NHCOCH2
244		NH2	2-thienyl	neopentyl	NHCOCH2
245		NH2	Pr	neopentyl	NHCOCH2
246		NH2	F	neopentyl	NHCOCH2
247		NH2	Cl	neopentyl	NHCOCH2
248		NH2	Br	neopentyl	NHCOCH2
249		NH2	Me	neopentyl	NHCOCH2
250		NH2	Et	neopentyl	CH2OCH2
251		NH2	SEt	neopentyl	CH2OCH2
252		NH2	SPr	neopentyl	CH2OCH2
253		NH2	2-furanyl	neopentyl	CH2OCH2
254		NH2	2-thienyl	neopentyl	CH2OCH2
255		NH2	Pr	neopentyl	CH2OCH2
256		NH2	F	neopentyl	CH2OCH2
257		NH2	Cl	neopentyl	CH2OCH2
258		NH2	Br	neopentyl	CH2OCH2
259		NH2	Me	neopentyl	CH2OCH2
260		NHBn	H	neopentyl	2,5-furanyl
261		NHPh	H	neopentyl	2,5-furanyl
262		NHBn	SMe	neopentyl	2,5-furanyl
263		NHPh	SMe	neopentyl	2,5-furanyl
264		NHPh-4-F	H	neopentyl	2,5-furanyl
265		NHPh-4-F	SMe	neopentyl	2,5-furanyl
266		NHNH2	F	neopentyl	2,5-furanyl
267		NH2	Me	cyclopropylmethyl	2,5-furanyl
268		NH2	SMe	cyclopropylmethyl	2,5-furanyl

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Table Compound No.	Synthetic Example No.				
269		NH2	F	cyclopropylmethyl	2,5-furanyl
270		NH2	Cl	cycloproprylmethyl	2,5-furanyl
271		NH2	Br	cyclopropylmethyl	2,5-furanyl
272		NH2	Et	cyclopropylmethyl	2,5-furanyl
273		NH2	CN	cyclopropylmethyl	2,5-furanyl
274		NH2	Me	cyclopropylmethyl	CONHCH2
275		NH2	SMe	cyclopropylmethyl	CONHCH2
276		NH2	F	cyclopropylmethyl	CONHCH2
277		NH2	Cl	cycloproprylmethyl	CONHCH2
278		NH2	Br	cyclopropylmethyl	CONHCH2
279		NH2	Et	cyclopropylmethyl	CONHCH2
280		NH2	CN	cyclopropylmethyl	CONHCH2
281		NH2	Me	cyclopropylmethyl	NHCOCH2
282		NH2	SMe	cyclopropylmethyl	NHCOCH2
283		NH2	F	cyclopropylmethyl	NHCOCH2
284		NH2	Cl	cycloproprylmethyl	NHCOCH2
285		NH2	Br	cyclopropylmethyl	NHCOCH2
286		NH2	Et	cyclopropylmethyl	NHCOCH2
287		NH2	CN	cyclopropylmethyl	NHCOCH2
288	2.18	NH2	H	3-(1-imidazolylpropyl)	2,5-furanyl
289	19.1	NH2	H	neopentyl	1,2-C6H4-O-
290	21.1	NH2	H	2-phenethyl	CONHCH2

More preferred are the following compounds from Table 1 including salts and prodrugs thereof:

42

1, 21, 22, 23, 29, 50, 57, 61, 62, 63, 66, 67, 72, 73, 89, 90, 107, 110, 111, 112, 113, 114,

115, 119, 123, 125, 126, 129, 130, 131, 132, 133, 134, 136, 137, 145, 148, 149, 151,

152, 153, 154, 155, 156, 158, 159, 163, 165, 173, 174, 175, 180, 181, 182, 183, 209,

210, 212, 215, 216, 217, 219, 220, 221, 230, 231, 234, 236, 237, 238, 240, 241, 246,

5   247, 248, 250, 251, 256, 257, 258, 266, 268, 269, and 272.

Most preferred are the following compounds and their salts and prodrugs:

N^9-neopentyl-2-methylthio-8-phosphonomethylaminocarbonyladenine;

N^9-neopentyl-2-methylthio-8-(2-(5-phosphono)furanyl)adenine;

N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine;

10   N^9-isobutyl-2-methylthio-8-(2-(5-phosphono)furanyl)adenine;

N^9-isobutyl-8-(2-(5-phosphono)furanyl)adenine;

N^9-cyclopropyl-8-(2-(5-phosphono)furanyl)adenine;

N^9-(2-cyclohexyl)ethyl-8-(2-(5-phosphono)furanyl)adenine;

N^9-(1-(2,2-dimethyl-3-chloro)propyl)-8-(2-(5-phosphono)furanyl)adenine;

15   N^9-(1-(3,3-dimethyl)butyl)-8-(2-(5-phosphono)furanyl)adenine;

N^9-(1,5,5-trimethyl-3-cyclohexen-1-yl)methyl-8-(2-(5-phosphono)furanyl)adenine;

N^9-neopentyl-8-(2-phosphonoacetylene-1-yl)adenine;

N^9-neopentyl-8-(1-(3-phosphono-3-sulfuryl)propyl)adenine;

N^9-neopentyl-8-(1-(3-phosphono-3-carboxyl)propyl)adenine;

20   N^9-neopentyl-8-(1-(3,3-diphosphono)propyl)adenine;

N^9-neopentyl-2-chloro-8-(2-(5-phosphono)furanyl)adenine;

2-Ethyl-N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine;

2-Methylthio-N^9-isobutyl-8-(2-(5-phosphono)furanyl)adenine; and

2-Methylthio-N^9-isobutyl-8-(phosphonomethoxymethyl)adenine.

25   Synthesis of Compounds of Formula 1

Synthesis of compounds encompassed by the present invention typically

includes some or all of the following general steps: (1) preparation of phosphonate

prodrug; (2) deprotection of phosphonate ester; (3) modification of C-8-substituted

purine intermediates; (4) modification of purine at positions other than C-8; (5)
construction of the purine ring system; and (6) preparation of 4,5-diaminopyrimidine
and other coupling partners.

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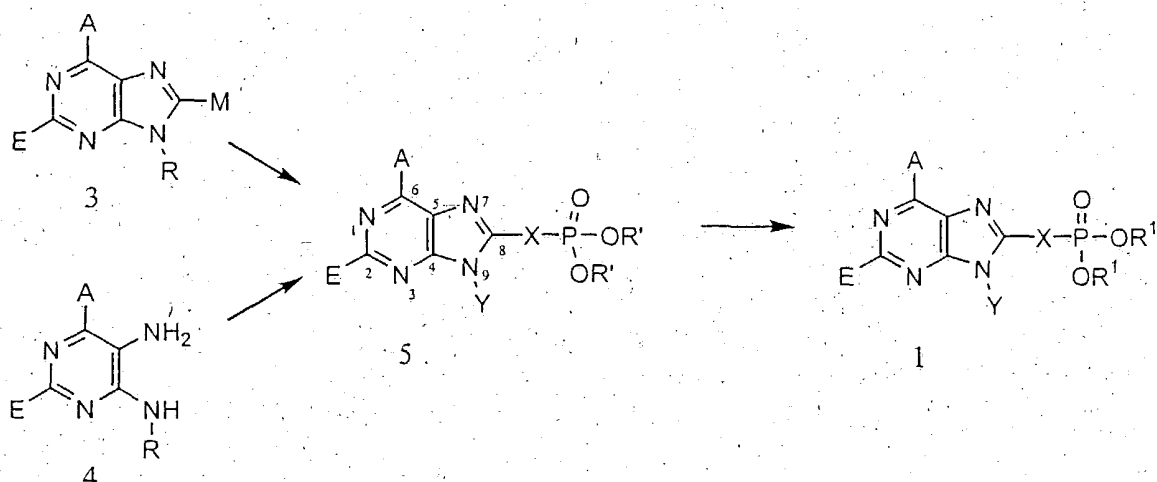
(1) Preparation of phosphonate prodrugs

     Prodrug esters can be introduced at different stages of the synthesis. Because of
their lability, prodrugs are often prepared from compounds of formula 1 where R^1 is H.
Advantageously, these prodrug esters can be introduced at an early stage, provided that
10   it can withstand the reaction conditions of the subsequent steps.

     Compounds of formula 5 where R^1 is H, can be alkylated with electrophiles
(such as alkyl halides, alkyl sulfonates etc) under nucleophilic substitution reaction
conditions to give phosphonate esters. For example prodrugs of formula 1, where R^1 is
acyloxymethyl group can be synthesized through direct alkylation of the free
15   phosphonic acid of formula 5, with the desired acyloxymethyl halide (e.g. Cl, Br, I;
Elhaddadi, et al *Phosphorus Sulfur*, 1990, *54(1-4)*: 143; Hoffmann, *Synthesis*, 1988, 62)
in presence of base e.g. *N, N'*-dicyclohexyl-4-morpholinecarboxamidine, Hunigs base
etc. in polar aprotic solvents such as DMF (Starrett, et al, J. Med. Chem., 1994, 1857).
These carboxylates include but not limited to acetate, propylate, isobutyrate, pivalate,
20   benzoate, and other carboxylates. Alternately, these acyloxymethylphosphonate esters

can also be synthesized by treatment of the nitrophosphonic acid (A is NO_2 in formula

5; Dickson, et al, *J. Med. Chem.*, 1996, *39*: 661; Iyer, et al, *Tetrahedron Lett.*, 1989, *30*:

7141; Srivastva, et al, *Bioorg. Chem.*, 1984, 12: 118). This can be extended to many

other types of prodrugs, such as compounds of formula 1 where R^1 is 3-phthalidyl, 2-

5   oxo-4,5-didehydro-1,3-dioxolanemethyl, and 2-oxotetrahydrofuran-5-yl groups, etc.

(Biller and Magnin (US 5,157,027); Serafinowska et al. (J. Med. Chem. *38*: 1372

(1995)); Starrett et al. (J. Med. Chem. *37*: 1857 (1994)); Martin et al. J. Pharm. Sci. 76:

180 (1987); Alexander et al., Collect. Czech. Chem. Commun, *59*: 1853 (1994)); and

EPO 0632048A1). N,N-Dimethylformamide dialkyl acetals can also be used to alkylate

10   phosphonic acids (Alexander, P., et al *Collect. Czech. Chem. Commun.*, 1994, *59*,

1853).

Alternatively, these phosphonate prodrugs or phosphoramidates can also be

synthesized, by reaction of the corresponding dichlorophosphonates and an alcohol or

an amine (Alexander, et al, *Collect. Czech. Chem. Commun.*, 1994, *59*: 1853). For

15   example, the reaction of dichlorophosphonate with phenols and benzyl alcohols in the

presence of base (such as pyridine, triethylamine, etc) yields compounds of formula 1

where R^1 is aryl (Khamnei, S., et al *J. Med. Chem.*, 1996, *39*: 4109; Serafinowska, H.T.,

et al *J. Med. Chem.*, 1995, *38*: 1372; De Lombaert, S., et al *J. Med. Chem.*, 1994, *37*:

498) or benzyl (Mitchell, A.G., et al *J. Chem. Soc. Perkin Trans. 1*, 1992, *38*: 2345).

20   The disulfide-containing prodrugs, reported by Puech et al., *Antiviral Res.*, 1993, *22*:

155, can also be prepared from dichlorophosphonate and 2-hydroxyethyl disulfide

under the standard conditions.

Such reactive dichlorophosphonate intermediates, can be prepared from the

corresponding phosphonic acids and the chlorinating agents e.g. thionyl chloride

25   (Starrett, et al, *J. Med. Chem.*, 1994, 1857), oxalyl chloride (Stowell, et al, *Tetrahedron*

Lett., 1990, *31*: 3261), and phosphorus pentachloride (Quast, et al, *Synthesis*, 1974,

490). Alternatively, these dichlorophosphonates can also be generated from disilyl

phosphonate esters (Bhongle, et al, *Synth. Commun.*, 1987, *17*: 1071) and dialkyl

45

phosphonate esters (Still, et al, *Tetrahedron Lett.*, 1983, *24*: 4405; Patois, et al, *Bull. Soc. Chim. Fr.*, 1993, *130*: 485).

Furthermore, these prodrugs can be prepared from Mitsunobu reactions (Mitsunobu, *Synthesis*, 1981, 1; Campbell, *J.Org. Chem.*, 1992, *52*: 6331), and other

5 acid coupling reagents include, but not limited to, carbodiimides (Alexander, et al, *Collect. Czech. Chem. Commun.*, 1994, *59*: 1853; Casara, et al, *Bioorg. Med. Chem. Lett.*, 1992, *2*: 145; Ohashi, et al, *Tetrahedron Lett.*, 1988, *29*: 1189), and benzotriazolyloxytris-(dimethylamino)phosphonium salts (Campagne, et al, *Tetrahedron Lett.*, 1993, *34*: 6743). The prodrugs of formula 1 where R^1 is the cyclic

10 carbonate or lactone or phthalidyl can also be synthesized by direct alkylation of free phosphonic acid with desired halides in the presence of base such as NaH or diisopropylethylamine (Biller and Magnin US 5,157,027; Serafinowska et al. J. Med. Chem. *38*: 1372 (1995); Starrett et al. J. Med. Chem. *37*: 1857 (1994); Martin et al. *J. Pharm. Sci. 76*: 180 (1987); Alexander et al., Collect. Czech. Chem. Commun, *59*: 1853

15 (1994); and EPO 0632048A1).

R^1 can also be introduced at an early stage of synthesis, when feasible. For example, compounds of formula 1 where R^1 is phenyl can be prepared by phosphorylation of 2-furanylpurines via strong base treatment (e.g. LDA) followed by chlorodiphenylphosphonate, as shown in the following scheme. Alternatively, such

20 compounds can be prepared by cyclization of 5-diphenylphosphono-2-furaldehyde with 4,5-diaminopyrimidines as described in section 5.
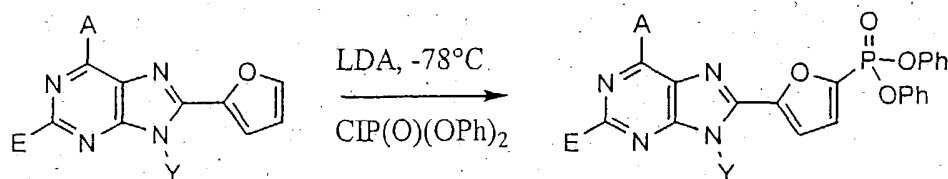
It is envisioned that compounds of formula 1 can be mixed phosphonate esters by combining the above described prodrugs (e.g. phenyl benzyl phosphonate esters,

25 phenyl acyloxyalkyl phosphonate esters, etc.). For example, the chemically combined

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phenyl-benzyl prodrugs are reported by Meier et al. *Bioorg. Med. Chem. Lett.*, 1997, 7:
99.

The substituted cyclic propyl phosphonate esters of formula 5, can be
synthesized by reaction of the corresponding dichlorophosphonate and the substituted
5   1,3-propanediol. The following are some of the methods to prepare the substituted 1,3-
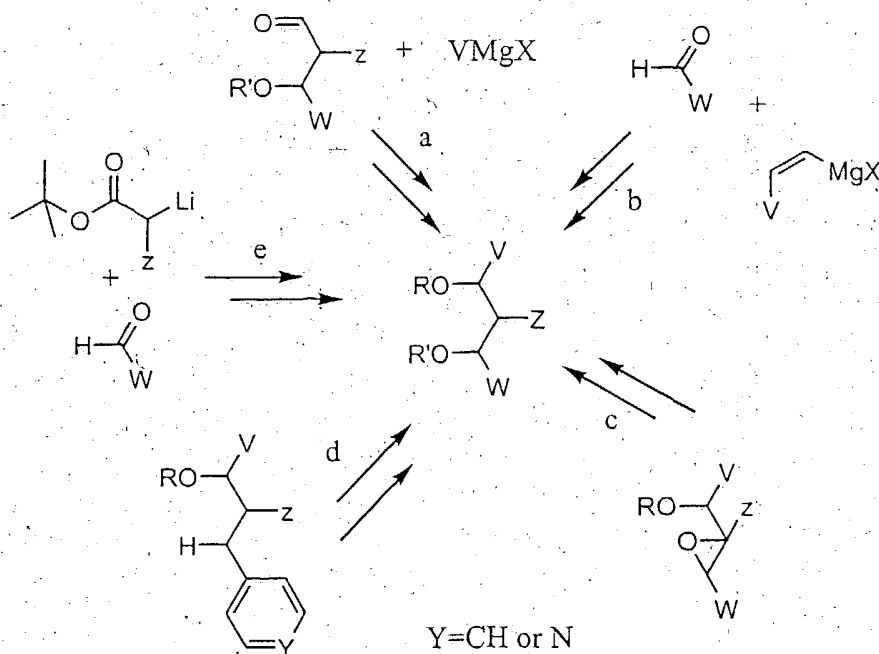propanediols.

Synthesis of the 1,3-Propanediols Used in the Preparation of Certain Prodrugs

The discussion of this step includes various synthetic methods for the
preparation of the following types of propane-1,3-diols: i) 1-substituted; ii) 2-
10   substituted; and iii) 1,2- or 1,3-annulated. Different groups on the prodrug part of the
molecule *i.e.*, on the propanediol moiety can be introduced or modified either during the
synthesis of the diols or after the synthesis of the prodrugs.

i) 1-Substituted 1,3-Propanediols

Propane-1,3-diols can be synthesized by several well known methods in the
15   literature. Aryl Grignard additions to 1-hydroxypropan-3-al gives 1-aryl-substituted
propane-1,3-diols (path a). This method will enable conversion of various substituted
aryl halides to 1-arylsubstituted-1,3-propanediols (Coppi, et. al., *J. Org. Chem.*, 1988,
53, 911). Aryl halides can also be used to synthesize 1-substituted propanediols by
Heck coupling of 1,3-diox-4-ene followed by reduction and hydrolysis (Sakamoto, et.
20   al., *Tetrahedron Lett.*, 1992, 33, 6845). A variety of aromatic aldehydes can be
converted to 1-substituted-1,3-propanediols by vinyl Grignard addition followed by
hydroboration (path b). Substituted aromatic aldehydes are also utilized by lithium-t-
butylacetate addition followed by ester reduction (path e) (Turner., *J. Org. Chem.*, 1990,
55 4744). In another method, commercially available cinnamyl alcohols can be
25   converted to epoxy alcohols under catalytic asymmetric epoxidation conditions. These
epoxy alcohols are reduced by Red-Al to result in enantiomerically pure propane-1,3-
diols (path c). Alternatively, enantiomerically pure 1,3-diols can be obtained by chiral
borane reduction of hydroxyethyl aryl ketone derivatives (Ramachandran, et. al.,

Tetrahedron Lett., **1997**, *38* 761). Pyridyl, quinoline, and isoquinoline propan-3-ol derivatives can be oxygenated to 1-substituted propan-1,3-diols by N-oxide formation followed by rearrangement under acetic anhydride conditions (path d) (Yamamoto, et. al., *Tetrahedron*, **1981**, *37*, 1871).

Y=CH or N

ii) 2-Substituted 1,3-Propanediols:

Various 2-substituted propane-1,3-diols can be made from commercially available 2-(hydroxymethyl)-1,3-propanediol. Triethyl methanetricarboxylate can be converted to the triol by complete reduction (path a) or diol-monocarboxylic acid derivatives can be obtained by partial hydrolysis and diester reduction (Larock, *Comprehensive Organic Transformations*, VCH, New York, **1989**). Nitrotriol is also known to give the triol by reductive elimination (path b) (Latour, et. al., *Synthesis*, **1987**, *8*, 742). The triol can be derivatized as a mono acetate or carbonate by treatment with alkanoyl chloride, or alkylchloroformate, respectively (path d) (Greene and Wuts, *Protective Groups in Organic Synthesis*, John Wiley, New York, **1990**). Aryl substitution can be made by oxidation to the aldehyde followed by aryl Grignard

additions (path c) and the aldehyde can also be converted to substituted amines by reductive amination reactions (path e).

Y=CH or N
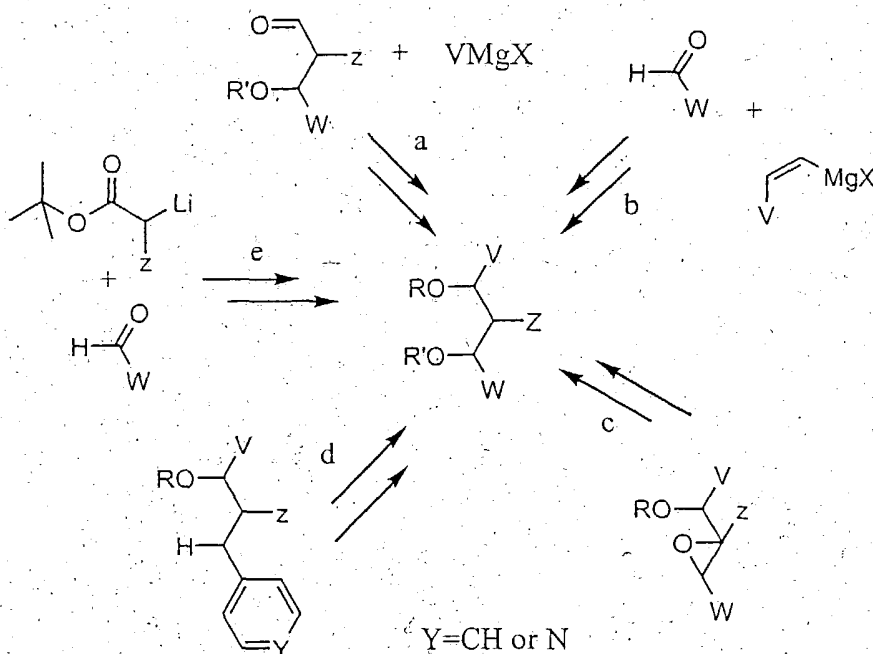
iii) Annulated 1,3-Propanediols:

5   Prodrugs of formula 1 where V - Z or V - W are fused by three carbons are made from cyclohexanediol derivatives. Commercially available cis, cis-1,3,5-cyclohexanetriol can be used for prodrug formation. This cyclohexanetriol can also be modified as described in the case of 2-substituted propane-1,3-diols to give various analogues. These modifications can either be made before or after formation of prodrugs. Various 1,3-cyclohexanediols can be made by Diels-Alder methodology

10   using pyrone as the diene (Posner, et. al., *Tetrahedron Lett.*, **1991**, 32, 5295). Cyclohexyl diol derivatives are also made by nitrile oxide olefin-additions (Curran, et. al., *J. Am. Chem. Soc.*, **1985**, 107, 6023). Alternatively, cyclohexyl precursors can be made from quinic acid (Rao, et. al., *Tetrahedron Lett.*, **1991**, 32, 547.)

(2) Deprotection of Phosphonate Esters

      Compounds of formula 1 where R^1 is H may be prepared from phosphonate esters using known phosphate and phosphonate ester cleavage conditions. For example, alkyl phosphonate esters are generally cleaved by reaction with silyl halides followed

5 by hydrolysis of the intermediate silyl phosphonate esters. Depending on the stability of the products, these reactions are usually accomplished in the presence of acid scavengers such as 1,1,1,3,3,3-hexamethyldisilazane, 2,6-lutidine, etc. Various silyl halides can be used for this transformation, such as chlorotrimethylsilane (Rabinowitz *J. Org. Chem.*, 1963, *28*: 2975), bromotrimethylsilane (McKenna et al. *Tetrahedron Lett.*,

10 1977, 155), iodotrimethylsilane (Blackburn et al. *J. Chem. Soc., Chem. Commun.*, 1978, 870). Phosphonate esters can also be cleaved under strong acid conditions, such as hydrogen halides in acetic acid or water, and metal halides (Moffatt et al. *U.S. Patent 3,524,846*, 1970). Phosphonate esters can also be converted to dichlorophosphonates with halogenating agents (e.g. PCl_5, $SOCl_2$, BBr_3, etc. Pelchowicz et al. *J. Chem. Soc.*,

15 1961, 238) and subsequently hydrolyzed to give phosphonic acids. Reductive reactions are useful in cleaving aryl and benzyl phosphonate esters. For example, aryl and benzyl phosphonate esters can be cleaved under hydrogenolysis conditions (Lejczak et al. *Synthesis*, 1982, 412; Elliott et al. *J. Med Chem.*, 1985, 28: 1208.) or dissolving metal reduction conditions (Shafer et al. *J. Am. Chem. Soc.*, 1977, *99*: 5118). (Elliott et al. *J.*

20 *Med. Chem.*, 1985, 28: 1208). Electrochemical (Shono et al. *J. Org. Chem.*, 1979, *44*: 4508) and pyrolysis (Gupta et al. *Synth. Commun.*, 1980, *10*: 299) conditions have also been used to cleave various phosphonate esters.

(3) Modification of C-8-substituted Purine Intermediates

      8-Substituted purines are useful intermediates in the preparation of compounds

25 of formula 1. 8-Halopurines, which are particularly useful intermediates, are readily prepared using chemistry well described in the literature. For example, N^9-alkyladenines are halogenated at the C-8 position using known halogenating agents (e.g. Br_2, NBS). 8-Alkylpurine can be prepared through direct lithiation of purine followed

by trapping with electrophiles (e.g. alkyl halides, Barton et al. *Tetrahedron Lett.*, 1979, 5877).

Functionaliztion of 8-halopurines can be accomplished under substitution reaction conditions with nucleophiles such as amines, alcohols, azides, sulfides, and 5 alkylthiols. It is advantageous to have the phosphonate moiety as part of the nucleophiles. For example, substitution of 8-bromopurine with aminoalkylphosphonates gives compounds of formula 1 where X is alkylamino.

8-Halopurines can also be transformed into other 8-substituted purines using palladium catalyzed reactions (Heck *Palladium Reagents in Organic Synthesis*; 10 Academic Press: San Diego, 1985). For example, palladium catalyzed carbonylation reactions of 8-bromopurine in the presence of alcohol gives 8-alkoxycarbonylpurines. Using known chemistry, the 8-carboxylate group can be converted into other functional groups, such as hydroxymethyl, halomethyl, formyl, carboxylic acid, carbamoyl, and thiocarbonyl groups. These functional groups are useful intermediates for the synthesis 15 of compounds of formula 1. For example, 8-alkyl and 8-arylpurines can be prepared from 8-halopurines via palladium catalyzed coupling reactions with organotin (Moriarty et al. *Tetrahedron Lett.*, 1990, *41*: 5877), organoborane (Yatagai, *Bull. Chem. Soc. Jpn.*, 1980, *53*: 1670), and other reagents known to couple with aryl halides. When the coupling reagents contain the dialkylphosphonate group, the reaction is useful for 20 preparation of compounds of formula 5 where X is alkyl, alkenyl, alkynyl, and aryl. For example, 8-bromopurine can be coupled with diethyl 1-tributylstannyl-3-allylphosphonate to give compounds of formula 5 where X is $-CH=CHCH_2-$. Subsequent hydrogenation reaction gives compounds of formula 5 where X is -$CH_2CH_2CH_2-$.

25 The phosphonate group can also be introduced by further modification of the 8-substituents. Substitutions of 8-haloalkyl or 8-sulfonylalkylpurine with nucleophiles containing the phosphonate group are useful for the preparation of compounds of formula 5 where X is alkylaminoalkyl, alkoxyalkyl, and alkylthioalkyl. For example,

51

compounds of formula 5 where X is -CH$_2$OCH$_2$- can be prepared from 8-bromomethylpurine using hydroxymethylphosphonate esters and a suitable base. It is possible to reverse the nature of the nucleophiles and electrophiles for the substitution reactions, i.e. haloalkyl- and/or sulfonylalkylphosphonate esters can be substituted with

5   purines containing a nucleophile at the C-8 position (such as 8-hydroxyalkyl, 8-thioalkyl, and 8-aminoalkylpurines). For example, diethyl phosphonomethyltriflate can be substituted by alcohols such as 8-hydroxymethylpurine to give compounds of formula 5 where X is -CH$_2$OCH$_2$- (Phillion et al. *Tetrahedron Lett.* 1986, *27*: 1477). Known amide formation reactions are useful for the synthesis of compounds of formula

10   5 where X is alkylaminocarbonyl, alkoxycarbonyl, alkoxythiocarbonyl, and alkylthiocarbonyl. For example, coupling of 8-purinecarboxylic acids with aminoalkylphosphonate esters gives compounds of formula 5 where X is alkylaminocarbonyl. For compounds of formula 5 where X is alkyl, the phosphonate group can also be introduced using other common phosphonate formation methods,

15   such as Michaelis-Arbuzov reaction (Bhattacharya et al. *Chem. Rev.*, 1981, *81*: 415), Michaelis-Becker reaction (Blackburn et al. *J. Organomet. Chem.*, 1988, *348*: 55), addition reactions of phosphorus to electrophiles (such as aldehydes, ketones, acyl halides, imines and other carbonyl derivatives).

         8-Azidopurines are useful for the preparation for compounds of formula 5 where

20   X is alkylamino and alkylcarbonylamino groups. For example, carboxylic acids (e.g. (RO)$_2$P(O)-alkyl-CO$_2$H) can be directly coupled to 8-azidopurines to give 8-alkylcarbonylaminopurines (Urpi et al. *Tetrahedron Lett.*, 1986, *27*: 4623). Alternatively, 8-azidopurines can also be converted to 8-aminopurines under reductive conditions, and subsequently converted to 8-alkylaminocarbonyl- and 8-

25   alkylaminopurines using known chemistry.

(4) Modification of purines at positions other than C-8

         Compounds of formula 5 can be further modified to give intermediates useful for the synthesis of compounds of formula 1. For example, substitution reactions of 6-

chloropurine by ammonia or alkylamines are useful for the preparations of compounds of formula 5 where A is amino and alkylamino groups.

E groups can be introduced by modifying existing 2-substituents of purine. For example, 2-halopurines, readily accessible from 2-aminopurines via chemistry well

5　described in the literature, can be converted to other 2-substituted purines by, for example, nucleophilic substitution reactions; transition metal catalyzed reactions, etc. (*J. Med. Chem.*, 1993, *36*: 2938; *Heterocycles*, 1990, *30*: 435).

E groups can also be introduced via metalation (e.g. lithiation, *J. Org. Chem.*, 1997, *62(20)*, 6833) of the C-2-position and followed by addition of electrophiles which

10　can be the desired E group or a substituent (e.g. tributylstannyl group) which can be converted to the desired E group using conventional chemistry.

It is envisioned that N^9-substituted purines can be readily prepared from compounds of formula 5 where Y is H using, for example, standard alkylation reactions (with alkyl halide, or sulfonate), or Mitsunobu reactions. Further elaborations of

15　substituents on Y are also possible.

More importantly, combinatorial methods have been developed for synthesis of 2- and N-9-substituted purines on solid-phase which conceivably can be applied for the synthesis of purine FBPase inhibitors (Schultz, et al, *Tetrahedron Lett.*, 1997, *38(7)*, 1161; *J. Am. Chem. Soc.*, 1996, *118*, 7430).

20　(5) Construction of the Purine Ring System

The purine ring system of compounds of formula 1 can be constructed using 4,5-diaminopyrimidines and carboxylates or their derivatives (such as aldehydes, amides, nitriles, ortho esters, imidates, etc.) (Townsend *Chemistry of Nucleosides and Nucleotides, Vol 1* ; Plenum Press, New York and London, page 156-158). For example,

25　alkyl and aryl aldehydes can be cyclized with 4,5-diaminopyrimidines as shown below.
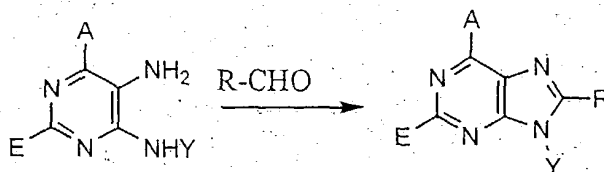
Intramolecular cyclization reactions of pyrimidine derivatives can also be used to construct the purine ring system. For example, 5-acylamino-4-alkylaminopyrimidines are treated with phosphorus oxychloride and cyclized under basic conditions to give purine derivatives. This transformation can also be achieved using other reagents (e.g. SiCl$_4$-Et$_3$N, Desaubry et al. *Tetrahedron Lett.*, 1995, *36*: 4249). Imidazole derivatives are also useful for the construction of purine ring system via cyclization reactions to form the pyrimidine ring (Townsend *Chemistry of Nucleosides and Nucleotides, Vol 1*; Plenum Press, New York and London, page 148-156).

(6) Preparation of Diaminopyrimidine and Other Coupling Partners

Compounds of formula 4 are useful for the construction of purine ring systems, and such compounds can be readily synthesized using known chemistry. For example, the Y group can be introduced using a nucleophilic substitution reaction involving an amine and 4-halopyrimidines (*Tetrahedron*, 1984, *40*: 1433). Alternatively, palladium catalyzed reactions (Wolfe et al. *J. Am. Chem. Soc.*, 1996, *118*: 7215) can also be used. Reductive amination reactions (*Synthesis*, 1975, 135) and alkylation with electrophiles (such as halides, sulfonates) are useful for the preparation of compounds of formula 4 from 4-aminopyrimidines. The 5-amino group can be introduced using amine formation reactions such as nitration followed by reduction (Dhainant et al. *J. Med. Chem.*, 1996, *39*: 4099), arylazo compound formation followed by reduction (Lopez et al. *Nucleosides & Nucleotides*, 1996, *15*: 1335), azide formation followed by reduction, or by rearrangement of carboxylic acid derivatives (e.g. Schmidt, Curtius, and Beckmann reactions).

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Coupling of aromatic or aliphatic aldehydes, and carboxylic acid derivatives with attached phosphonate esters are particularly suited for the preparation of compounds of formula 1 as described in section 5. Such phosphonate esters are prepared by lithiation of the aromatic ring using methods well described in literature

5   (Gschwend *Org. React.* 1979, *26*: 1) followed by addition of phosphorylating agents (e.g. $ClPO_3R_2$). Phosphonate esters can also be introduced by Arbuzov-Michaelis reaction (Brill *Chem Rev.*, 1984, *84*: 577) and transition metal catalyzed reaction with alkyl halides and aryl halides or triflates (Balthazar et al. *J. Org. Chem.*, 1980, *45*: 5425; Petrakis et al. *J. Am. Chem. Soc.*, 1987, *109*: 2831; Lu et al. *Synthesis*, 1987, 726).

10   Alternatively, aryl phosphonate esters can be prepared from aryl phosphates under anionic rearrangement conditions (Melvin *Tetrahedron Lett.*, 1981, *22*: 3375; Casteel et al. *Synthesis*, 1991, 691). Aryl phosphate esters can also be used to prepare compounds of Formula 1 where X is an oxyaryl group. N-Alkoxy aryl salts with alkali metal derivatives of dialkyl phosphonate can be used to synthesize heteroaryl-2-phosphonate

15   esters (Redmore *J. Org. Chem.*, 1970, *35*: 4114).

A second lithiation step can be used to incorporate the aldehyde functionality, although other methods known to generate aromatic aldehydes can be envisioned as well (e.g. Vilsmeier-Hack reaction, Reimar-Teimann reaction etc.). In the second lithiation step, the lithiated aromatic ring is treated with reagents that either directly

20   generate an aldehyde (e.g. DMF, HCO_2R, etc.) or with reagents that lead to a group that subsequently is transformed into an aldehyde group using known chemistry (e.g. alcohol, ester, cyano, alkene, etc.). It is also envisioned that the sequence of these reactions can be reversed, i.e. the aldehyde moiety can be incorporated first followed by the phosphorylation reaction. The order of the reaction will depend on the reaction

25   conditions and the protecting groups. Prior to the phosphorylation, it is also envisioned that it may be advantageous to protect the aldehydes using a number of well-known steps (hemiacetal, hemiaminal, etc.,). The aldehyde is then unmasked after

phosphorylation. (*Protective groups in Organic Synthesis, Greene*, T. W., 1991, Wiley,

New York).

Formulations

5   Compounds of the invention are administered orally in a total daily dose of about 0.1 mg/kg/dose to about 100 mg/kg/dose, preferably from about 0.3 mg/kg/dose to about 30 mg/kg/dose. The most preferred dose range is from 0.5 to 10 mg/kg (approximately 1 to 20 nmoles/kg/dose). The use of time-release preparations to control the rate of release of the active ingredient may be preferred. The dose may be administered in as many divided doses as is convenient. When other methods are used

10   (e.g. intravenous administration), compounds are administered to the affected tissue at a rate from 0.3 to 300 nmol/kg/min, preferably from 3 to 100 nmoles/kg/min. Such rates are easily maintained when these compounds are intravenously administered as discussed below.

      For the purposes of this invention, the compounds may be administered by a

15   variety of means including orally, parenterally, by inhalation spray, topically, or rectally in formulations containing pharmaceutically acceptable carriers, adjuvants and vehicles. The term parenteral as used here includes subcutaneous, intravenous, intramuscular, and intraarterial injections with a variety of infusion techniques. Intraarterial and intravenous injection as used herein includes administration through catheters. Oral

20   administration is generally preferred.

      Pharmaceutical compositions containing the active ingredient may be in any form suitable for the intended method of administration. When used for oral use for example, tablets, troches, lozenges, aqueous or oil suspensions, dispersible powders or granules, emulsions, hard or soft capsules, syrups or elixirs may be prepared.

25   Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents and preserving agents, in order to provide a palatable preparation. Tablets

containing the active ingredient in admixture with non-toxic pharmaceutically

acceptable excipient which are suitable for manufacture of tablets are acceptable. These

excipients may be, for example, inert diluents, such as calcium or sodium carbonate,

lactose, calcium or sodium phosphate; granulating and disintegrating agents, such as

5   maize starch, or alginic acid; binding agents, such as starch, gelatin or acacia; and

lubricating agents, such as magnesium stearate, stearic acid or talc. Tablets may be

uncoated or may be coated by known techniques including microencapsulation to delay

disintegration and adsorption in the gastrointestinal tract and thereby provide a

sustained action over a longer period. For example, a time delay material such as

10   glyceryl monostearate or glyceryl distearate alone or with a wax may be employed.

          Formulations for oral use may be also presented as hard gelatin capsules where

the active ingredient is mixed with an inert solid diluent, for example calcium phosphate

or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water

or an oil medium, such as peanut oil, liquid paraffin or olive oil.

15   Aqueous suspensions of the invention contain the active materials in admixture

with excipients suitable for the manufacture of aqueous suspensions. Such excipients

include a suspending agent, such as sodium carboxymethylcellulose, methylcellulose,

hydroxypropyl methylcelluose, sodium alginate, polyvinylpyrrolidone, gum tragacanth

and gum acacia, and dispersing or wetting agents such as a naturally occurring

20   phosphatide (e.g., lecithin), a condensation product of an alkylene oxide with a fatty

acid (e.g., polyoxyethylene stearate), a condensation product of ethylene oxide with a

long chain aliphatic alcohol (e.g., heptadecaethyleneoxycetanol), a condensation

product of ethylene oxide with a partial ester derived from a fatty acid and a hexitol

anhydride (e.g., polyoxyethylene sorbitan monooleate). The aqueous suspension may

25   also contain one or more preservatives such as ethyl or n-propyl p-hydroxy-benzoate,

one or more coloring agents, one or more flavoring agents and one or more sweetening

agents, such as sucrose or saccharin.

Oil suspensions may be formulated by suspending the active ingredient in a

vegetable oil, such as arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil

such as liquid paraffin. The oral suspensions may contain a thickening agent, such as

beeswax, hard paraffin or cetyl alcohol. Sweetening agents, such as those set forth

5   above, and flavoring agents may be added to provide a palatable oral preparation.

These compositions may be preserved by the addition of an antioxidant such as ascorbic

acid.

Dispersible powders and granules of the invention suitable for preparation of an

aqueous suspension by the addition of water provide the active ingredient in admixture

10   with a dispersing or wetting agent, a suspending agent, and one or more preservatives.

Suitable dispersing or wetting agents and suspending agents are exemplified by those

disclosed above. Additional excipients, for example sweetening, flavoring and coloring

agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of

15   oil-in-water emulsions. The oily phase may be a vegetable oil, such as olive oil or

arachis oil, a mineral oil, such as liquid paraffin, or a mixture of these. Suitable

emulsifying agents include naturally-occurring gums, such as gum acacia and gum

tragacanth, naturally occurring phosphatides, such as soybean lecithin, esters or partial

esters derived from fatty acids and hexitol anhydrides, such as sorbitan monooleate, and

20   condensation products of these partial esters with ethylene oxide, such as

polyoxyethylene sorbitan monooleate. The emulsion may also contain sweetening and

flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, such as glycerol,

sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative, a

25   flavoring or a coloring agent.

The pharmaceutical compositions of the invention may be in the form of a

sterile injectable preparation, such as a sterile injectable aqueous or oleaginous

suspension. This suspension may be formulated according to the known art using those

suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally acceptable diluent or solvent; such as a solution in 1,3-butane-diol or prepared as a lyophilized powder. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile fixed oils may conventionally be employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid may likewise be used in the preparation of injectables.

The amount of active ingredient that may be combined with the carrier material to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. For example, a time-release formulation intended for oral administration to humans may contain 20 to 2000 μmol (approximately 10 to 1000 mg) of active material compounded with an appropriate and convenient amount of carrier material which may vary from about 5 to about 95% of the total compositions. It is preferred that the pharmaceutical composition be prepared which provides easily measurable amounts for administration. For example, an aqueous solution intended for intravenous infusion should contain from about 0.05 to about 50 μmol (approximately 0.025 to 25 mg) of the active ingredient per milliliter of solution in order that infusion of a suitable volume at a rate of about 30 mL/hr can occur.

As noted above, formulations of the present invention suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be administered as a bolus, electuary or paste.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a

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suitable machine the active ingredient in a free flowing form such as a powder or

granules, optionally mixed with a binder (e.g., povidone, gelatin, hydroxypropylmethyl

cellulose), lubricant, inert diluent, preservative, disintegrant (e.g., sodium starch

glycolate, cross-linked povidone, cross-linked sodium carboxymethyl cellulose) surface

5   active or dispersing agent. Molded tablets may be made by molding in a suitable

machine a mixture of the powdered compound moistened with an inert liquid diluent.

The tablets may optionally be coated or scored and may be formulated so as to provide

slow or controlled release of the active ingredient therein using, for example,

hydroxypropyl methylcellulose in varying proportions to provide the desired release

10   profile. Tablets may optionally be provided with an enteric coating, to provide release

in parts of the gut other than the stomach. This is particularly advantageous with the

compounds of formula 1 when such compounds are susceptible to acid hydrolysis.

Formulations suitable for topical administration in the mouth include lozenges

comprising the active ingredient in a flavored base, usually sucrose and acacia or

15   tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and

glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a

suitable liquid carrier.

Formulations for rectal administration may be presented as a suppository with a

suitable base comprising for example cocoa butter or a salicylate.

20   Formulations suitable for vaginal administration may be presented as pessaries,

tampons, creams, gels, pastes, foams or spray formulations containing in addition to the

active ingredient such carriers as are known in the art to be appropriate.

Formulations suitable for parenteral administration include aqueous and non-

aqueous isotonic sterile injection solutions which may contain antioxidants, buffers,

25   bacteriostats and solutes which render the formulation isotonic with the blood of the

intended recipient; and aqueous and non-aqueous sterile suspensions which may include

suspending agents and thickening agents. The formulations may be presented in unit-

dose or multi-dose sealed containers, for example, ampoules and vials, and may be

stored in a freeze-dried (lyophilized) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

5   Preferred unit dosage formulations are those containing a daily dose or unit, daily sub-dose, or an appropriate fraction thereof, of a fructose 1,6-bisphosphatase inhibitor compound.

It will be understood, however, that the specific dose level for any particular patient will depend on a variety of factors including the activity of the specific

10   compound employed; the age, body weight, general health, sex and diet of the individual being treated; the time and route of administration; the rate of excretion; other drugs which have previously been administered; and the severity of the particular disease undergoing therapy, as is well understood by those skilled in the art.

Utility

15   FBPase inhibitors at the AMP site may be used to treat diabetes mellitus, lower blood glucose levels, and inhibit gluconeogenesis.

FBPase inhibitors at the AMP site may also be used to treat excess glycogen storage diseases. Excessive hepatic glycogen stores are found in patients with some glycogen storage diseases. Since the indirect pathway contributes significantly to

20   glycogen synthesis (Shulman, G.I. *Phys. Rev.* 1992. 72, 1019-1035), inhibition of the indirect pathway (gluconeogenesis flux) is expected to decrease glycogen overproduction.

FBPase inhibitors at the AMP site may also be used to treat or prevent diseases associated with increased insulin levels.

25   Increased insulin levels are associated with an increased risk of cardiovascular complications and atherosclerosis (Folsom, et al., *Stroke*, 1994, 25, 66-73; Howard, G. et al., *Circulation* 1996, 93, 1809-1817). FBPase inhibitors are expected to decrease postprandial glucose levels by enhancing hepatic glucose uptake. This effect is

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postulated to occur in individuals that are non-diabetic (or pre-diabetic, i.e. without

elevated HGO or fasting blood glucose levels). Increased hepatic glucose uptake will

decrease insulin secretion and thereby decrease the risk of diseases or complications

that arise from elevated insulin levels.

5   The compounds of this invention and their preparation can be understood further

by the examples which illustrate some of the processes by which these compounds are

prepared. These examples should not, however, be construed as specifically limiting

the invention and variations of the invention, now known or later developed, are

considered to fall within the scope of the present invention as hereinafter claimed.

10   **EXAMPLES**

Example 1

Preparation of 5-diethylphosphono-2-furaldehyde (1).

Step A.   A solution of 2-furaldehyde diethyl acetal (1 mmol) in THF was treated

with nBuLi (1 mmol) at -78 °C. After 1 h, diethyl chlorophosphate (1.2 mmol) was

15   added and the reaction was stirred for 40 min. Extraction and evaporation gave a brown

oil.

Step B.   The resulting brown oil was treated with 80 % acetic acid at 90 °C for

4 h. Extraction and chromatography gave compound 1 as a clear yellow oil.

          Alternatively this aldehyde can be prepared from furan as described below:

20   Step C.   A solution of furan (1 mmol) in diethyl ether was treated with TMEDA

(N,N,N'N'-tetramethylethylenediamine) (1 mmol) and nBuLi (2 mmol) at -78 °C. The

solution was stirred for 0.5 h. at -78 °C and diethyl chlorophosphate was added and

stirred for another 1 h. Extraction and distillation produced diethyl 2-furanphosphonate

as a clear oil.

25   Step D.   A solution of diethyl 2-furanphosphonate (1 mmol) in THF

(tetrahydrofuran) was treated with LDA (1.12 mmol, lithium N,N-diisopropylamide) at

-78 °C for 20 min. Methyl formate (1.5 mmol) was added and the reaction was stirred for 1 h. Extraction and chromatography gave compound 1 as a clear yellow oil.

Preferably this aldehyde can be prepared from 2-furaldehyde as described below.

5   Step E.   A solution of 2-furaldehyde (1 mmol) and N,N'-dimethylethylene diamine (1 mmol) in toluene was refluxed with a Dean-Stark trap to collect the resulting water. After 2 h the solvent was removed in vacuo and the residue was distilled to give furan-2-(N,N'-dimethylimidazolidine) as a clear colorless oil, bp 59-61 °C (3 mm Hg).

Step F.   A solution of furan-2-(N,N'-dimethylimidazolidine) (1 mmol) and

10   TMEDA (1 mmol) in THF was treated with nBuLi (1.3 mmol) at -40 to -48 °C. The reaction was stirred at 0 °C for 1.5 h and then cooled to -55 °C and treated with a solution of diethylchlorophosphate (1.1 mmol) in THF. After stirring at 25 °C for 12 h the reaction mixture was evaporated and subjected to extraction to give 5-diethylphosphono-furan-2-(N,N'-dimethylimidazolidine) as a brown oil.

15   Step G.   A solution of 5-diethylphosphonofuran-2-(N,N'-dimethyl-imidazolidine) (1 mmol) in water was treated with concentrated sulfuric acid until pH = 1. Extraction and chromatography gave compound 1 as a clear yellow oil.

Example 2.

Preparation of N^9-substituted-8-(2-(5-phosphono)furanyl)adenines.

20   The preparation of N^9-(2-phenethyl)-8-(2-(5-phosphono)furanyl)adenine is given as an example:

Step A.   A solution of 5-amino-4,6-dichloropyrimidine (1 mmol) in nBuOH was treated with Et$_3$N (1.2 mmol) and phenethylamine (1.05 mmol) at 80 °C. After 12 h, the cooled reaction mixture was evaporated under vacuum and the residue was

25   chromatographed to give 6-chloro-5-amino-4-(phenethylamino)-pyrimidine as a yellow solid. mp 156 - 157 °C; TLC: R$_f$ = 0.41, 50 % EtOAc-hexane.

Step B.   The 6-chloro-5-amino-4-(2-phenethylamino)pyrimidine (1 mmol) in DMSO was treated with 2-furaldehyde (1.5 mmol) and FeCl$_3$-silica (2.0 mmol) at

80 °C. After 12 h, the cooled reaction mixture was filtered and the filtrate was

evaporated under vacuum. Chromatography afforded 6-chloro-N^9-(2-phenethyl)-8-(2-

furanyl)purine as a yellow solid. TLC: Rf= 0.62, 50 % EtOAc-hexane. Anal. calcd. for

$C_{17}H_{13}N_4OCl$: C: 62.87; H: 4.03; N: 17.25. Found: C: 62.66; H: 3.96; N: 17.07.

5   Step C.   The 6-chloro-N^9-(2-phenethyl)-8-(2-furanyl)purine (1 mmol) in THF

was treated with LDA (1.5 mmol) at -78 °C. After 1 h, diethyl chlorophosphate (5

mmol) was added and the reaction was stirred at -78 °C for 2 h and then quenched with

saturated NH$_4$Cl. Extraction and chromatography gave 6-chloro-N^9-(2-phenethyl)-8-(2-

(5-diethylphosphono)-furanyl)purine as a yellow solid. TLC: Rf= 0.34, 100 % EtOAc.

10   Alternatively this type of compound can be prepared as follows:

Step D.   A solution of 6-chloro-5-amino-4-(2-phenethylamino)pyrimidine (1

mmol) in DMSO was treated with 5-diethylphosphono-2-furaldehyde (1, 1.5 mmol),

and FeCl$_3$-silica (2.0 mmol) at 80 °C. After 12 h., the cooled reaction mixture was

filtered and the filtrate was evaporated under vacuum. Chromatography afforded 6-

15 chloro-N^9-(2-phenethyl)-8-(2-(5-diethyl-phosphono)furanyl)purine as a yellow solid.

TLC: Rf= 0.34, 100 % EtOAc.

Step E.   6-Chloro-N^9-(2-phenethyl)-8-(2-(5-diethylphosphono)furanyl)-purine

(1 mmol) in THF-DMSO was treated with liquid ammonia (2 mL) in a steel bomb.

After 12 h, the reaction was evaporated under vacuum and the residue was purified

20 through chromatography to give N^9-(2-phenethyl)-8-(2-(5-

diethylphosphono)furanyl)adenine as a yellow solid. TLC: Rf= 0.12, 5 % MeOH -

CH$_2$Cl$_2$.

Step F.   A solution of N^9-(2-phenethyl)-8-(2-(5-diethylphosphono)furanyl)-

adenine (1 mmol) in acetonitrile was treated with bromotrimethylsilane (10 mmol).

25 After 12 h, the reaction was evaporated under vacuum and the residue was treated with

a mixture of water and acetonitrile. The solid was collected through filtration to give

N^9-(2-phenethyl)-8-(2-(5-phosphono)furanyl)adenine (2.1). mp 242-244° C; Anal.

calcd. for $C_{17}H_{16}N_5O_4P + 1.37H_2O$: C: 50.16; H: 4.64; N: 17.21. Found: C: 48.95; H: 4.59; N: 16.80.

The following compounds were prepared according to above procedures:

2.2: N^9-(2-cyclohexylethyl)-8-(2-(5-phosphono)furanyl)adenine. mp 194-195 °C ;

5   Anal. calcd. for $C_{17}H_{22}N_5O_4P + 1\ H_2O$: C: 49.90; H: 5.90; N: 17.10. Found: C: 50.20; H: 5.70; N: 17.10.

2.3: N^9-(2-naphthylmethyl)-8-(2-(5-phosphono)furanyl)adenine. mp 255-256 °C ; Anal. calcd. for $C_{20}H_{16}N_5O_4P + 1\ H_2O$: C: 54.70; H: 4.10; N: 15.90. Found: C: 54.30; H: 4.20; N: 15.90.

10   **2.4:** N^9-(1-(2,2-diphenyl)ethyl)-8-(2-(5-phosphono)furanyl)adenine. mp 220-221 °C ; Anal. calcd. for $C_{23}H_{20}N_5O_4P + 0.25\ H_2O$: C: 59.29; H: 4.43; N: 15.03. Found: C: 59.35; H: 4.25; N: 14.83.

2.5: N^9-ethyl-8-(2-(5-phosphono)furanyl)adenine. mp > 230 °C ; Anal. calcd. for $C_{11}H_{12}N_5O_4P + 1\ H_2O$: C: 40.38; H: 4.31; N: 21.40. Found: C: 40.45; H: 4.18; N: 21.44.

15   **2.6:** N^9-isobutyl-8-(2-(5-phosphono)furanyl)adenine. mp > 230 °C ; Anal. calcd. for $C_{13}H_{16}N_5O_4P$: C: 46.30; H: 4.78; N: 20.76. Found: C: 46.00; H: 4.61; N: 20.49.

2.7: N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine. mp > 230 °C ; Anal. calcd. for $C_{14}H_{18}N_5O_4P$: C: 47.87; H: 5.16; N: 19.94. Found: C: 47.59; H: 4.92; N: 19.53.

2.8: N^9-adamentanemethyl-8-(2-(5-phosphono)furanyl)adenine. mp > 250 °C ; Anal.

20   calcd. for $C_{20}H_{24}N_5O_4P + 0.5\ H_2O + 0.25\ MeOH$: C: 54.48; H: 5.87; N: 15.69. Found: C: 54.62; H: 5.52; N: 15.36.

2.9: N^9-cyclopropyl-8-(2-(5-phosphono)furanyl)adenine. mp > 250 °C ; MS (M + H) calcd for $C_{12}H_{13}N_5O_4P$: 322, found: 322.

2.10: N^9-cyclopentyl-8-(2-(5-phosphono)furanyl)adenine. mp 220 °C (decomp); Anal.

25   calcd. for $C_{14}H_{16}N_5O_4P + 1\ H_2O$: C: 45.78; H: 4.94; N: 19.07. Found: C: 45.40; H: 4.79; N: 18.73.

2.11: N^9-((2-ethoxy)phenyl)methyl-8-(2-(5-phosphono)furanyl)-adenine. mp > 230 °C ;
Anal. calcd. for $C_{18}H_{18}N_5O_5P + 2\ H_2O$: C: 47.90; H: 4.91; N: 15.52. Found: C: 48.03;
H: 4.53; N: 15.25.

2.12: N^9-(1-(3-N,N-dimethylamino-2,2-dimethyl)propyl)-8-(2-(5-phosphono)-
furanyl)adenine. mp > 230 °C ; Anal. calcd. for $C_{16}H_{23}N_6O_4P + 3\ H_2O + 0.5\ HOAc + $
$0.75\ Na$: C: 41.19; H: 6.30; N: 16.95. Found: C: 41.35; H: 6.04; N: 16.57.

2.13: N^9-(1-(3-hydroxyl-2,2-dimethyl)propyl)-8-(2-(5-phosphono)-furanyl)adenine. mp
> 230 °C ; Anal. calcd. for $C_{14}H_{18}N_5O_5P + 0.25\ H_2O$: C: 45.23; H: 5.02; N: 18.83.
Found: C: 45.40; H: 5.02; N: 18.44.

2.14: N^9-(1-(3-chloro-2,2-dimethyl)propyl)-8-(2-(5-phosphono)-furanyl)adenine. mp >
230 °C ; Anal. calcd. for $C_{14}H_{17}N_5O_4PCl + 0.125\ CHCl_3 + 0.06\ AcOEt$: C: 42.50;
H: 4.37; N: 17.25. Found: C: 42.62; H: 3.99; N: 16.87.

2.15: N^9-(1-(3,3-dimethyl)butyl)-8-(2-(5-phosphono)furanyl)-adenine. mp 230 °C ;
Anal. calcd. for $C_{15}H_{20}N_5O_4P + 1.25H_2O + 0.13\ AcOEt$: C: 46.68; H: 5.94; N: 17.56.
Found: C: 46.67; H: 5.78; N: 17.35.

2.16: N^9-(1,5,5-trimethyl-3-cyclohexenyl)methyl-8-(2-(5-phosphono)furanyl)-adenine.
mp > 230 °C ; Anal. calcd. for $C_{19}H_{24}N_5O_4P + 0.5\ H_2O + 0.13\ AcOEt$: C: 53.54; H:
5.99; N: 16.01. Found: C: 53.67; H: 5.69; N: 15.75.

2.17: N^9-(1-(1,2,2-trimethyl)propyl)-8-(2-(5-phosphono)-furanyl)adenine. mp > 250 °C;
Anal. calcd. for $C_{15}H_{20}N_5O_4P + 0.67\ H_2O + 0.13AcO\ Et$: C: 47.74; H: 5.70; N: 18.56.
Found: C: 47.99; H: 5.39; N: 18.49.

2.18: 6-Amino-9-(3-(1-imidazolyl)propyl)-8-(2-(5-phosphono)furanyl)purine. mp 182-
186 °C; Mass calcd. for $C_{15}H_{16}N_7O_4P$: 389. Found: $M + H^+ = 390$.

Examples 3.

Preparation of N^9-substituted-8-(2-phosphonoethylamino)adenines.

Step A.   Adenine (1 mmol) in DMF was treated with sodium hydride (1.2 mmol)
followed by benzyl bromide (1.2 mmol) at room temperature under nitrogen. The

resulting mixture was warmed at 100 °C for 2 h. The cooled reaction mixture was

evaporated to dryness. Extraction and chromatography afforded N^9-benzyladenine.

Step B.   A solution of N^9-benzyladenine (1 mmol) in acetic acid buffer (pH = 4)

was treated with bromine (1 mmol) at room temperature for 12 h. The reaction was

quenched with 10 % sodium sulfite solution and extracted with dichloromethane. The

combined extracts were dried (Na_2SO_4) and evaporated to dryness. Chromatography

afforded N^9-benzyl-8-bromoadenine.

Step C.   A mixture of N^9-benzyl-8-bromoadenine (1 mmol),

aminoethylphosphonate (2 mmol), and sodium hydroxide (2 mmol) in ethanol-water in

a sealed tube was warmed at 110 °C under nitrogen. After 24 h the cooled reaction

mixture was purified through preparative HPLC to give N^9-benzyl-8-(2-

phosphonoethylamino)adenine (3.1). Exact mass calculated for $C_{14}H_{17}N_6O_3P + H^+$:

349.1178. Found: 349.1180.

The following compounds were prepared according to this procedure:

3.2: N^9-phenethyl-8-(2-phosphonoethylamino)adenine. mp 159-160 °C ; Anal. calcd.

for $C_{15}H_{19}N_6O_3P + 1.25 H_2O$: C: 46.81; H: 5.63; N: 21.84. Found: C: 47.05; H: 5.63;

N: 21.48.

3.3: N^9-(2-naphthylmethyl)-8-(2-phosphonoethylamino)adenine. mp 189-190 °C ;

Anal. calcd. for $C_{18}H_{19}N_6O_3P + 1.5 H_2O$: C: 50.82; H: 5.21; N: 19.76. Found: C: 50.71;

H: 5.25; N: 19.54.

3.4: N^9-cyclohexylethyl-8-(2-phosphonoethylamino)adenine. mp > 250 °C ; Anal.

calcd. for $C_{15}H_{25}N_6O_3P + 0.33 H_2O$: C: 48.12; H: 6.91; N: 22.44. Found: C: 48.12;

H: 6.78; N: 22.15.

Example 4.

Preparation of 8-(2-phosphonoethylamino)adenosines.

A mixture of 8-bromoadenosine (1 mmol), aminoethylphosphonate (2 mmol),

and sodium hydroxide (2 mmol) in ethanol-water in a sealed tube was warmed at

110 °C under nitrogen. After 24 h the cooled reaction mixture was purified through

preparative HPLC to give 8-(2-phosphonoethylamino)-adenosine (4.1). mp 175 °C ;
Anal. calcd. for $C_{12}H_{19}N_6O_7P$ + 0.5 H_2O: C: 36.10; H: 5.05; N: 21.05; P: 7.76. Found:
C: 36.08; H: 4.83; N: 20.36; P: 7.86.

The following compound was prepared in this manner:

5   4.2:   8-(2-Phosphonoethylamino)-5-deoxyadenosine as a white solid. mp 220 °C ;
Anal. calcd. for $C_{12}H_{19}N_6O_6P$ + 1.5 H_2O: C: 35.92; H: 5.53; N: 20.94. Found: C: 36.15;
H: 5.12; N: 20.53.

Examples 5.

Preparation of N^9-alkyl-8-(phosphonomethoxymethyl)adenines.

10   Step A.   A mixture of N^9-phenethyl-8-bromoadenine (1 mmol), tetrakis
(triphenylphosphine)palladium (0.05 mmol), and triethylamine (5 mmol) in DMF in a
sealed tube was warmed at 110 °C under 50 psi of carbon monoxide. After 24 h the
cooled reaction mixture was evaporated and purified through chromatography to gave
N^9-phenethyl-8-methoxycarbonyladenine as a yellow solid. TLC: Rf= 0.12, 5 %

15   MeOH-CH_2Cl_2.

Step B.   A solution of N^9-phenethyl-8-methoxycarbonyladenine (1 mmol) in
tetrahydrofuran was treated with lithium aluminum hydride (1 mmol) at 0 °C for 1 h.
Extraction and chromatography gave N^9-phenethyl-8-hydroxymethyl-adenine as a white
solid. TLC: Rf= 0.31, 10 % MeOH-CH_2Cl_2.

20   Step C.   A solution of N^9-phenethyl-8-hydroxymethyladenine (1 mmol) in
methylene chloride was treated with phosphorus tribromide (1 mmol) at 25 °C for 1 h.
Extraction and chromatography gave N^9phenethyl-8-bromomethyl-adenine as a white
solid. TLC: R_f = 0.31, 10 % MeOH-CH_2Cl_2.

Step D.   A solution of N^9-phenethyl-8-bromomethyladenine (1 mmol) in DMF
25   was treated with a solution of diethyl hydroxymethylphosphonate sodium salt (1 mmol)
in DMF at 25 °C for 1 h. Extraction and chromatography gave N^9-phenethyl-8-
diethylphosphonomethoxymethyladenine as a white solid. TLC: R_f = 0.31, 10 %
MeOH-CH_2Cl_2.

N^9-phenethyl-8-diethylphosphonomethoxymethyladenine was subjected to Step

F in Example 2 to give N^9-(2-phenethyl)-8-(phosphonomethoxymethyl)-adenine (5.1)

as a white solid. mp > 250 °C ; Anal. calcd. for $C_{19}H_{22}N_5O_4P$ + 0.75 H_2O: C: 56.93; H:

5.91; N: 10.48. Found: C: 56.97; H: 5.63; N: 10.28.

5   The following compounds were prepared according to this procedure:

5.2: N^9-(2-cyclohexylethyl)-8-(phosphonomethoxymethyl)adenine. mp > 250 °C ;

Anal. calcd. for $C_{15}H_{24}N_5O_4P$ + 1 H_2O: C: 46.51; H: 6.76; N: 18.08. Found: C: 46.47;

H: 6.71; N: 17.91.

5.3: N^9-(1-nanonyl)-8-(phosphonomethoxymethyl)adenine. mp 195-210 °C ; Anal.

10   calcd. for $C_{16}H_{28}N_5O_4P$ + 1 H_2O: C: 47.64; H: 7.50; N: 17.36. Found: C: 47.33; H: 7.34;

N: 16.99.

5.4: N^9-(3-cyclohexylpropyl)-8-(phosphonomethoxymethyl)adenine. mp 230-

250 °C; Anal. calcd. for $C_{19}H_{22}N_5O_4P$ + 0.9 H_2O + 0.3 HBr: C: 45.34; H: 6.68; N:

16.52. Found: C: 45.74; H: 6.39; N: 16.18.

15   Alternatively this type of compound can also be prepared according to the

following procedure:

Step E.   A solution of 6-chloro-5-amino-4-(neopentylamino)pyrimidine (1 mmol)

in diethyl ether was treated with pyridine (3 mmol), and acetoxyacetyl chloride (1.2

mmol) at 25 °C for 12 h. Extraction and chromatography afforded 6-chloro-5-

20   acetoxyacetyl-amino-4-neopentylaminopyrimidine as a yellow solid. TLC: R_f = 0.18,

30 % EtOAc-hexane.

Step F.   A solution of 6-chloro-5-acetoxyacetylamino-4-neopentyl-

aminopyrimidine (1 mmol) in phosphorus oxychloride was heated at reflux for 6 h. The

cooled reaction mixture was evaporated to dryness and the residue was dissolved in

25   pyridine and stirred at 25 °C for 20 h. Evaporation and chromatography afforded 6-

chloro-8-acetoxymethyl-N^9-neopentylpurine as a yellow solid. TLC: R_f = 0.51, 50 %

EtOAc-hexane.

Step G. A solution of 6-chloro-8-acetoxymethyl-N^9-neopentylpurine (1 mmol) in THF-water was treated with aqueous sodium hydroxide (1.5 mmol) at 0 °C for 0.5 h. Extraction and chromatography afforded 6-chloro-8-hydroxymethyl-N^9-neopentylpurine as a yellow gel. TLC: R$_f$ = 0.38, 33 % EtOAc-hexane.

5 Step H. A solution of 6-chloro-8-hydroxymethyl-N^9-neopentylpurine (1 mmol) in methylene chloride was treated with phosphorus tribromide (1 mmol) at 25 °C for 6 h. Extraction and chromatography afforded 6-chloro-8-bromomethyl-N^9-neopentylpurine as a white solid. TLC: R$_f$ = 0.64, 25 % EtOAc-hexane.

Step I. A solution of 6-chloro-8-bromomethyl-N^9-neopentylpurine (1 mmol) in 10 DMF was treated with a solution of sodium diethylphosphono-methoxide (1 mmol) at 25 °C for 6 h. Extraction and chromatography afforded 6-chloro-8-diethyl-phosphonomethoxymethyl-N^9-neopentylpurine as a white solid. TLC: R$_f$ = 0.31, 50 % EtOAc-hexane.

Step J. A solution of 6-chloro-8-diethylphosphonomethoxymethyl-N^9-15 neopentylpurine (1 mmol) in THF-DMSO was treated with liquid ammonia (10 mmol) at 25 °C for 6 h. Extraction and chromatography afforded 8-diethylphosphonomethoxymethyl-N^9-neopentyladenine as a white solid. TLC: R$_f$ = 0.44, 25 % MeOH-EtOAc.

8-Diethylphosphonomethoxymethyl-N^9-neopentyladenine was subjected to Step 20 F in Example 2 to give N^9-neopentyl-8-(phosphonomethoxymethyl)-adenine (5.5) as a white solid. mp > 250 °C ; Anal. calcd. for C$_{12}$H$_{20}$N$_5$O$_4$P + 1.5 H$_2$O: C: 40.45; H: 6.51; N: 19.65. Found: C: 40.68; H: 6.35; N: 19.40.

Examples 6.

Preparation of N^9-substituted-8-(1-(3-phosphono)propyl)adenines.

25 Step A. A mixture of diethyl propargylphosphonate (1 mmol, prepared according to *J. Org. Chem.*, 1993, *58(24)*, 6531.), tributyltin hydride (1.05 mmol), and AIBN (0.005 mmol) was heated at 60 °C for 18 h. The cooled reaction mixture was purified

through chromatography to give dimethyl (1-tributylstannyl)allyl-3-phosphonate as a yellow oil.

Step B.   A solution of N^9-(2-cyclohexylethyl)-8-bromoadenine (1 mmol), tetrakis(triphenylphosphine)palladium (0.1 mmol), and dimethyl (1-

5   tributylstannyl)allyl-3-phosphonate (5 mmol) in DMF was warmed at 90 °C under nitrogen. After 2 h the cooled reaction mixture was evaporated and purified through chromatography to give N^9-(2-cyclohexylethyl)-8-(3-dimethylphosphonopropene-1-yl)adenine as a yellow solid. TLC: $R_f = 0.48$, 10 % MeOH - CH_2Cl_2.

Step C.   A solution of N^9-(2-cyclohexylethyl)-8-(3-dimethylphosphono-propene-

10   1-yl)adenine in methanol-acetic acid was stirred at room temperature under 50 psi of H_2 for 12 h. Filtration and chromatography afforded N^9-(2-cyclohexylethyl)-8-(1-(3-dimethylphosphono)propyl)adenine as a yellow solid. TLC: $R_f = 0.26$, 10 % MeOH - CH_2Cl_2.

N^9-(2-cyclohexylethyl)-8-(1-(3-dimethylphosphono)propyl)adenine was

15   subjected to Step F in Example 2 to give N^9-(2-cyclohexylethyl)-8-(1-(3-phosphono)propyl)adenine (6.1) as a white solid: mp 122-125 °C ; Anal. calcd. for $C_{16}H_{26}N_5O_3P$ + 0.25 AcOH: C: 51.83; H: 7.12; N: 18.31. Found: C: 51.87; H: 6.96; N: 17.96.

6.2: N^9-(2-phenethyl)-8-(1-(3-phosphono)propyl)adenine was also prepared in this

20   manner as a solid. mp > 250 °C Anal. calcd. for $C_{16}H_{20}N_5O_3P$ + 0.5 H_2O: C: 51.89; H: 5.71; N: 18.91. Found: C: 51.81; H: 5.49; N: 18.66.

Examples 7.

Preparation of N^9-(2-phenethyl)-8-(2-(5-phosphono)thienyl)adenine.

Step A.   A solution of 2-thienyllithium in THF (1 mmol) was added to a solution

25   of diethyl chlorophosphate (1 mmol) at -78 °C under nitrogen. After 2 h the reaction was warmed to room temperature and quenched with brine. Extraction and chromatography afforded 2-diethylphosphonothiophene as a yellow oil. TLC: $R_f = 0.37$, 50 % EtOAc - hexane.

<u>Step B.</u>   A solution of 2-diethylphosphonothiophene (1 mmol) in THF was treated with nBuLi at -78 °C for 1 h. Tributyltin chloride was added and stirred at -78 °C for 2 h and the reaction was quenched with water and warmed to room temperature. Extraction and chromatography afforded diethyl 2-(5-

5   tributylstannyl)thienylphosphonate as a yellow oil. TLC: R_f = 0.65, 50 % EtOAc - hexane.

<u>Step C.</u>   A mixture of N^9-phenethyl-8-bromoadenine (1 mmol), tetrakis (triphenylphosphine)palladium (0.1 mmol), and diethyl 2-(5-tributylstannyl)-thienylphosphonate (5 mmol) in DMF was warmed at 80 °C under nitrogen. After 21 h

10   the cooled reaction mixture was evaporated to dryness. The dark oil was triturated with hexane and the residue was dissolved in CH_2Cl_2 and filtered. The filtrate was evaporated to give N^9-(2-phenethyl)-8-(2-(5-diethylphosphono)-thienyl)adenine as a yellow solid. TLC: R_f = 0.50, 10 % MeOH - CH_2Cl_2.

        N^9-(2-phenethyl)-8-(2-(5-diethylphosphono)thienyl)adenine was subjected to

15   Step F in Example 2 to give N^9-(2-phenethyl)-8-(2-(5-phosphono)-thienyl)adenine (7.1) as a white solid. mp > 250 °C; Anal. calcd. for $C_{17}H_{16}N_5O_3SP$ + 0.5H_2O: C: 49.76; H: 4.17; N: 17.07. Found: C: 50.07; H: 4.02; N: 17.45.

        N^9-(2-phenethyl)-8-(2-(5-phosphono)thienyl)adenine can also be made via a cyclization reaction between 5-diethylphosphono-2-thiophene-carboxaldehyde

20   (prepared from 2-thienyllithium as described in Steps C and D of Example 1) as described in Example 2.

<u>Example 8.</u>

<u>Preparation of N^9-(2-cyclohexylethyl)-8-(phosphonomethylthio)-adenine.</u>

<u>Step A.</u>   A mixture of N^9-(2-cyclohexylethyl)-8-bromoadenine (1 mmol), and K_2S

25   (4 mmol) in ethanol was warmed at 110 °C for 7 h, and at 85 °C for 12 h. The cooled reaction mixture was filtered, evaporated and purified through chromatography to give N^9-(2-cyclohexylethyl)-8-thiohydroxyadenine as a yellow solid. TLC: R_f = 0.26, 5 % MeOH - CH_2Cl_2.

Step B.   A mixture of N^9-(2-cyclohexylethyl)-8-thiohydroxyadenine (1 mmol),

K_2CO_3 (4 mmol), and diethyl chloromethylphosphonate (3 mmol) in DMF was stirred

at room temperature for 48 h. Extraction and chromatography gave N^9-(2-

cyclohexylethyl)-8-diethylphosphono-methylthioadenine. TLC: $R_f = 0.35$, 10 % MeOH

5   - EtOAc.

N^9-(2-Cyclohexylethyl)-8-diethylphosphonomethylthioadenine was subjected to

Step F in Example 2 to give N^9-(2-cyclohexylethyl)-8-(phosphonomethylthio)adenine

(8.1) as a white solid. mp 240 - 243 °C ; Anal. Calcd. for $C_{14}H_{22}N_5O_3SP + 1.25H_2O$:

C: 42.69; H: 5.95; N: 17.54. Found: C: 42.62; H: 6.03; N: 17.80.

10   Example 9.

Preparation of 6-chloro-9-phenethyl-8-(2-(5-phosphono)furanyl)purine.

6-Chloro-N^9-phenethyl-8-(2-(5-diehtylphosphono)furanyl)purine (Step C in

Example 2) was subjected to procedure of Step F in Example 2 to give compound 9.1 as

a yellow solid. mp >200 °C ; Anal. calcd. for $C_{17}H_{14}N_4O_4PCl + 2 H_2O + 0.28$ HBr:

15   C: 44.06; H: 3.98; N: 12.09. Found: C: 43.86; H: 3.59; N: 12.02.

Example 10.

Preparation of N^6,N^9-substituted-8-(2-(5-phosphono)furanyl)adenines.

A solution of 6-chloro-N^9-substituted-8-(2-(5-diethylphosphono)furanyl)-purine

(1 mmol) in DMSO was treated with alkylamine at 100 °C for 12 h. Evaporation and

20   chromatography gave N^6,N^9-substituted-8-(2-(5-diethyl-phosphono)furanyl)adenines.

The title compounds were obtained by subjecting N^6,N^9-substituted-8-(2-(5-

diethylphosphono)furanyl)adenines to the procedure of Step F in Example 2.

The following compounds were prepared in this manner:

10.1: 6-Dimethylamino-N^9-phenethyl-8-(2-(5-phosphono)furanyl)purine as a white

25   solid. mp >200 °C ; Anal. calcd. for $C_{19}H_{20}N_5O_4P$: C: 55.2; H: 4.8; N: 16.9. Found: C:

54.9; H: 4.9; N: 16.6.

10.2: 6-Methylamino-N^9-phenethyl-8-(2-(5-phosphono)furanyl)purine as a white solid. mp 242 °C ; Anal. calcd. for $C_{18}H_{18}N_5O_4P$ + 1 H_2O: C: 51.8; H: 4.8; N: 16.8. Found: C: 51.7; H: 4.8; N: 16.7.

Example 11.

5   Preparation of 2-methylthio-6-amino-N^9-isobutyl-8-(2-(5-phosphono)furanyl)purine and 2-methylsulfonyl-6-amino-N^9-isobutyl-8-(2-(5-phosphono)furanyl)purine.

Step A: 2-Methylthio-4,5,6-triaminopyrimidine and 5-diethylphosphono-2-furaldehyde was subjected to the procedures of Step D in Example 2 to give 6-amino-2-methylthio-8-(2-(5-diethylphosphono)furanyl)purine as a yellow solid. TLC: R_f = 0.27,

10   80 % EtOAc - hexane.

Step B: 6-Amino-2-methylthio-8-(2-(5-diethylphosphono)furanyl)purine was alkylated with isobutyl bromide following the procedures of Step A in Example 3 to give 6-amino-N^9-isobutyl-2-methylthio-8-(2-(5-diethylphosphono)-furanyl)purine as a yellow solid. TLC: R_f = 0.27, 80 % EtOAc - hexane.

15   Step C: 6-Amino-N^9-isobutyl-2-methylthio-8-(2-(5-diethyl-phosphono)-furanyl)purine was subjected to Step F in Example 2 to give 6-amino-N^9-isobutyl-2-methylthio-8-(2-(5-phosphono)-furanyl)purine (**11.1**) as a white solid. mp 220 °C ; Anal. calcd. for $C_{14}H_{18}N_5O_4PS$ + 0.25 HBr + 0.25 EtOAc: C: 42.33; H: 4.8; N: 16.45. Found: C: 42.42; H: 4.53; N: 16.39.

20   Step D: A solution of 6-amino-N^9-isobutyl-2-methylthio-8-(2-(5-diethyl-phosphono)furanyl)purine (1 mmol) in 50 mL of methanol was cooled to 0 °C and treated with an acetone solution of Oxone (1.6 mmol). After stirring for 3 h at 25 °C the reaction was extracted and then chromatographed to give 6-amino-N^9-isobutyl-2-methylsulfonyl-8-(2-(5-diethylphosphono)furanyl)purine as a white solid. TLC: R_f =

25   0.24, 100 % EtOAc.

Step E: 6-Amino-N^9-isobutyl-2-methylsulfonyl-8-(2-(5-diethylphosphono)-furanyl)purine was subjected to Step F in Example 2 to give 6-amino-N^9-isobutyl-2-methylsulfonyl-8-(2-(5-phosphono)furanyl)purine (**11.2**) as a white solid. mp 240 °C

(decomp); Anal. calcd. for $C_{14}H_{18}N_5O_6PS$ + 0.5 H_2O: C: 39.62; H: 4.51; N: 16.5. Found: C: 39.77; H: 4.44; N: 16.12.

Example 12.

Preparation of 6-amino-N^9-neopentyl-8-(2-(3,4-dichloro-5-phosphono)furanyl)purine.

5   Step A: A solution of 3,4-dichloro-2-furoic acid (1 mmol) in diethyl ether was treated with LDA (3 mmol) at -78 °C for 30 min and then treated with diethyl chlorophosphate (3.5 mmol) at -78 °C for 1 h. The reaction was quenched and extracted to give 5-diethylphosphono-3,4-dichloro-2-furoic acid as a yellow foam.

Step B: A solution of 5-diethylphosphono-3,4-dichloro-2-furoic acid (1 mmol) in
10   methylene chloride was treated with oxalyl chloride and DMF at 25 °C for 1 h. The reaction mixture was evaporated and the residue was dissolved in diethyl ether and treated with a solution of 4-chloro-5-amino-6-neopentyl-aminopyrimidine (1 mmol) and pyridine (3 mmol) in diethyl ether at 25 °C for 16 h. Extraction and chromatography gave 4-chloro-5-(2-(3,4-dichloro-5-diethylphosphono)furoyl)amino-6-
15   neopentylaminopyrimidine as a yellow solid. TLC: R_f = 0.4, 50 % EtOAc - hexane.

Step C: A solution of 4-chloro-5-(2-(3,4-dichloro-5-diethylphosphono)-furoyl)amino-6-neopentylaminopyrimidine (1 mmol) in dichloromethane was treated with silicone tetrachloride (2.5 mmol) and triethylamine (2.5 mmol) at 45 °C for 18 h. The cooled reaction mixture was subjected to extraction and chromatography to give 6-
20   chloro-N^9-neopentyl-8-(2-(3,4-dichloro-5-diethyl-phosphono)furanyl)purine as a yellow solid. TLC: R_f = 0.28, 50 % EtOAc - hexane.

Step D: 6-Chloro-N^9-neopentyl-8-(2-(3,4-dichloro-5-diethylphosphono)-furanyl)purine was subjected to Steps E and F in Example 2 to give 6-amino-N^9-neopentyl-8-(2-(3,4-dichloro-5-phosphono)furanyl)purine (12.1) as a white solid. mp >
25   250 °C ; Anal. calcd. for $C_{14}H_{16}N_5O_4PCl_2$ + 0.5 H_2O + 0.15 EtOAc: C: 39.64; H: 4.15; N: 15.83. Found: C: 39.82; H: 3.88; N: 15.46.

Example 13.

Preparation of hydroxyethyldisulfidylethylphosphonate diester.

A suspension of 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (1 mmol) in

thionyl chloride (5 mL) was warmed at reflux for 4 h. The cooled reaction mixture was

evaporated to dryness and the resulting yellow residue was treated with a solution of 2-

hydroxyethyl disulfide (4 mmol), pyridine (2.5 mmol) in methylene chloride. After

stirring at 25 °C for 4 h the reaction was subjected to extraction and chromatography to

give two compounds:

13.1: N^9-phenethyl-8-(bis(6'-hydroxy-3',4'-disulfide)hexylphosphono)furanyl)-adenine.

Anal. calcd for $C_{25}H_{32}N_5O_6S_4P$ + 0.5 DMSO + 1.5 H_2O: C: 43.15; H: 5.29; N: 9.68.

Found: C: 43.38; H: 4.93; N: 9.34.

13.2: N^9-phenethyl-8-((3',4'-disulfide)nonacyclicphosphono)furanyladenine.

Anal. calcd for $C_{21}H_{22}N_5O_4S_2P$ + DMSO: C: 47.49; H: 4.85; N: 12.04. Found: C: 47.93;

H: 4.60; N: 11.76.

Example 14.

Preparation of substituted benzyl phosphonate diesters.

A suspension of 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (1 mmol) in

thionyl chloride (5 mL) was warmed at refluxing 4 h. The cooled reaction mixture was

evaporated to dryness and a solution of the resulting yellow residue was added to a

solution of the corresponding benzyl alcohol (4 mmol), and pyridine (2.5 mmol) in

methylene chloride. After stirring at 25 °C for 4 h the reaction mixture was subjected to

extraction and chromatography to give the title compounds.

14.1: N^9-phenethyl-8-(2-(5-(bis-(3-bromo-4-methoxy)benzyl)phosphono)-

furanyl)adenine. Molecular mass calculated for $C_{33}H_{30}N_5O_6Br_2P$ + H$^+$: 784. Found:

784.

14.2: N^9-phenethyl-8-(2-(5-(bis-(3-cyano-4-methoxy)benzyl)phosphono)-

furanyl)adenine. Anal. calcd. for $C_{35}H_{30}N_7O_6P$ + 0.5 H_2O : C: 61.40; H: 4.56; N: 14.32.

Found: C: 61.45; H: 4.51; N: 14.18.

14.3: N^9-neopentyl-8-(2-(5-(bis-(4-acetoxy)benzyl)phosphono)furanyl)adenine. Anal. calcd. for $C_{32}H_{34}N_5O_8P$ + 0.6 H_2O : C: 58.37; H: 5.39; N: 10.64. Found: C: 58.11; H: 5.28; N: 10.42.

N^9-neopentyl-8-(2-(5-(bis-(3-phthalidyl-2-ethyl)phosphono)furanyl)-adenine is

5   also prepared following the above described procedure using 2-(3-phthalidyl)ethanol which was prepared from phthalide-3-acetic acid in Example 27.

This reaction procedure can also be used to prepare diaryl ester prodrugs of phosphonates, such as substituted phenyl esters of phosphonate.

Example 15.

10   Preparation of 6-amino-8-(2-(5-diphenylphosphono)furanyl)-N^9-(2-phenyl)ethylpurine.

Step A.   A suspension of 6-chloro-8-(2-furanyl)-N^9-phenethylpurine (1 mmol) in THF at -78 °C was treated with LDA (1.3 mmol) for 1 h. Then a solution of diphenyl chlorophosphate in THF was added and the reaction was stirred at -78 °C for another hour. The reaction was warmed to 0 °C and quenched with aqueous saturated sodium

15   bicarbonate. Extraction and chromatography gave 6-chloro-8-(2-(5-diphenylphosphono)furanyl)-N^9-phenethylpurine as a white solid. mp 117-118 °C .

Step B.   A solution of 6-chloro-8-(2-(5-diphenylphosphono)furanyl)-N^9-phenethylpurine (1 mmol) in DMF was treated with sodium azide (4 mmol) and triphenylphosphine (4 mmol) at room temperature for 3 h. Filtration, evaporation of the

20   filtrate followed by chromatography gave 6-triphenyl-phosphonoimino-8-(2-(5-diphenylphosphono)furanyl)-N^9-(2-phenyl)ethylpurine as a beige foam.

Step C.   A solution of 6-triphenylphosphonoimino-8-(2-(5-diphenyl-phosphono)furanyl)-9-(2-phenyl)ethylpurine (1 mmol) in THF was treated with aqueous hydrogen chloride at room temperature for 24 h. Evaporation and chromatography gave

25   6-amino-8-(2-(5-diphenylphosphono)furanyl)-N^9-(2-phenyl)ethylpurine (15.1) as a pale yellow solid. mp 196-197 °C ; Anal. calcd. for $C_{29}H_{24}N_5O_4P$: C: 64.80; H: 4.50; N: 13.03; P: 5.76. Found: C: 64.50; H: 4.47; N: 12.98; P: 5.46.

Example 16.

Preparation of acyloxymethylphosphonate diesters.

A solution of 8-(2-(5-phosphono)furanyl)-N^9-phenethyladenine (1 mmol) in acetonitrile and N,N,N-diisopropylethylamine (5 mmol) was treated with

5   acyloxymethyl iodide (4 mmol) at 0 °C for 24 h. Extraction and chromatography gave the title compounds.

The following compounds were prepared according to this procedure:

16.1: 6-Amino-9-phenethyl-8-(2-(5-diisobutyrylmethylphosphono)furanyl)-purine. Anal. calcd for $C_{27}H_{32}N_5O_8P$: C: 55.40; H: 5.50; N: 12.00. Found: C: 55.60; H: 5.60; N:

10   11.80.

16.2: 6-Amino-9-(2-cyclohexylethyl)-8-(2-(5-diisobutyrylmethylphosphono)-furanyl)purine. Anal. calcd for $C_{27}H_{38}N_5O_8P$ + 0.7 H_2O: C: 53.70; H: 6.60; N: 11.60. Found: C: 54.00; H: 6.50; N: 11.20.

16.3: 6-Amino-9-ethyl-8-(2-(5-diisobutyrylmethylphosphono)-furanyl)purine.

15   Anal. calcd for $C_{21}H_{28}N_5O_8P$: C: 49.51; H: 5.54; N: 13.75. Found: C: 49.75; H: 5.37; N: 13.76.

16.4: 6-Amino-9-neopentyl-8-(2-(5-diisobutyrylmethylphosphono)furanyl)-purine. Anal. calcd for $C_{24}H_{34}N_5O_8P$: C: 52.27; H: 6.21; N: 12.70. Found: C: 52.40; H: 6.27; N: 12.41.

20   **16.5**: 6-Amino-9-neopentyl-8-(2-(5-dipivaloxymethylphosphono)furanyl)purine. Anal. calcd for $C_{26}H_{38}N_5O_8P$ + 0.2 EtOAc: C: 53.90; H: 6.68; N: 11.73. Found: C: 54.10; H: 6.80; N: 11.42.

6-Amino-9-phenethyl-8-(2-(5-bis-(3-(5,6,7-trimethoxy)phthalidyl)-phosphono)furanyl)purine (**16.6**) was also synthesized following this procedure using 3-

25   bromo-5,6,7-trimethoxyphthalide as the alkylating reagent to give the titled compound as a white solid after preparative HPLC purification. mp 155-160 °C ; Anal. calcd. for $C_{39}H_{36}N_5O_{14}P$ + H_2O: C: 55.26; H: 4.52; N: 8.26. Found: C: 54.89; H: 4.75; N: 8.21.

Example 17.

Preparation of 5-methyl-4-hydroxymethyl-2-oxo-1,3-dioxolene.

A solution of 4,5-dimethyl-2-oxo-1,3-dioxolene (1 mmol) and selenium dioxide (2.5 mmol) in dioxane was heated at reflux for 1 h. Evaporation, extraction and chromatography gave 5-methyl-4-hydroxymethyl-2-oxo-1,3-dioxolene as a yellow oil. TLC: $R_f = 0.5$, 5 % MeOH-dichloromethane.

Example 18.

Preparation of (5-substituted 2-oxo-1,3-dioxolen-4-yl)methyl phosphonate prodrugs.

A solution of N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (1 mmol) in DMF and 2 mmol of sodium hydride is treated with 5-methyl-4-bromomethyl-2-oxo-1,3-dioxolene (4 mmol, prepared according to *Chem. Pharm. Bull.* 1984, 32(6), 2241) at 25 °C for 24 h. Extraction and chromatography gives N^9-neopentyl-8-(2-(5-bis(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl phosphono)-furanyl)adenine.

Alternatively, N^9-neopentyl-8-(2-(5-bis(5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl phosphono)-furanyl)adenine is prepared from N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine and 5-methyl-4-hydroxymethyl-2-oxo-1,3-dioxolene (prepared from 4,5-dimethyl-2-oxo-1,3-dioxolene as described in Example 17) according to procedures of Example 14.

Example 19.

Preparation of 2-(6-amino-N^9-neopentylpurin-8-yl)phenyl phosphonate:

Step A.   Triethylamine (1.1 mmol) was added slowly to an ice-cooled solution of 6-chloro-N^9-neopentyl-8-(2-hydroxyphenyl)purine (1 mmol) and diethyl phosphite (1 mmol) in carbontetrachloride. The reaction was stirred at room temperature overnight. Triethylamine hydrochloride was precipitated as a white solid mass. Extraction and chromatography gave diethyl 2-(6-Chloro-N^9-neopentylpurin-8-yl)phenyl phosphate.

Step B.   Diethyl 2-(6-Chloro-N^9-neopentylpurin-8-yl)phenyl phosphate was subjected to Step E and F in Example 2 to give the title compound (19.1). mp > 250 °C;

Anal. calcd. for $C_{16}H_{20}N_5O_4P + 1.25\ H_2O$: C: 48.06; H: 5.67; N: 17.51. Found: C:48.42; H: 5.42; N: 17.15.

Example 20.

Preparation of N^9-neopentyl-8-(1-(2-phosphono)imidazolemethyl)adenine:

5   Step A.   A solution of 1-benzylimidazole (1.1 mmol) in THF was treated with LDA (1.1 mmol) at -78 °C for 1 h, and followed by addition of diethyl chlorophosphate (2 mmol), and stirred for 2 h. Extraction and chromatography gave 1-benzyl-2-diethylphosphonoimidazole as a yellow oil. TLC: R_f = 0.35, 80 % EtOAc-hexane.

       Step B.   A solution of 1-benzyl-2-diethylphosphonoimidazole (1 mmol) in EtOH

10   was treated with palladium on carbon (10 %) at 25 °C under 1 atmosphere of hydrogen for 19 h. Filtration and evaporation gave 2-diethyl-phosphonoimidazole as a white solid. TLC: R_f = 0.05, 80 % EtOAc-hexane.

       Step C.   A solution of 8-bromomethyl-6-chloro-N^9-neopentylpurine (1 mmol, Step H of Example 5), 2-diethylphosphonoimidazole (2.5 mmol), and N,N,N-

15   diisopropylethylamine (2.5 mmol) in acetonitrile was stirred at 25 °C for 48 h. Extraction and chromatography gave 6-chloro-N^9-neopentyl-8-(1-(2-diethylphosphono)imidazolemethyl)purine.

       Step D.   6-Chloro-N^9-neopentyl-8-(1-(2-diethylphosphono)imidazole-methyl)purine was subjected to Steps E and F in Example 2 to give the title compound

20   (20.1). mp > 250 °C ; MS (M + H) calcd. for $C_{14}H_{20}N_7O_3P$: 366; found: 366.

Example 21.

Preparation of N^9-phenethyl-8-(phosphonomethylaminocarbonyl)adenine:

       Step A. N^9-phenethyl-8-(methoxycarbonyl)adenine (1 mmol, prepared as in Step A of Example 5) was treated with sodium hydroxide (1.2 mmol) in THF:MeOH:H$_2$O

25   (3:2:1) at 25 °C for 1.5 h. The reaction mixture was evaporated to dryness, and the residue was dissolved in DMF, treated with diethyl aminomethylphosphonate (1.5 mmol), EDCl (1-(3-dimethyl-aminopropyl)-3-ethylcarbodiimide hydrochloride, 1.3

mmol), HOBt (1-hydroxy-benzotriazole hydrate, 1.5 mmol), and stirred at 25 °C for 24 h. Extraction and chromatography gave N^9-phenethyl-8-(diethylphosphonomethyl-aminocarbonyl)adenine as a white solid. TLC: $R_f = 0.1$, EtOAc.

Step B. N^9-phenethyl-8-(diethylphosphonomethylaminocarbonyl)adenine (1

5   mmol) was subjected to Step F in Example 2 to give the title compound (21.1). mp > 250 °C; Anal. calcd. for $C_{15}H_{17}N_6O_4P$ + 0.17 Toluene: C: 47.32; H: 4.50; N: 20.45. Found: C:47.67; H: 4.57; N: 20.78.

Example 22.

Preparation of 2-substituted N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine:

10   Step A.   A solution of 2-amino-4,6-dichloropyrmidine (1 mmol), neopentylamine (1.05 mmol), and triethylamine (2 mmol) in n-butanol was stirred at 110 °C for 12 h. Extraction and chromatography gave 2-amino-4-chloro-6-neopentylpyrimidine as a yellow solid. TLC: $R_f = 0.2$, 30 % EtOAc-hexane.

Step B.   A mixture of 2-amino-4-chloro-6-neopentylpyrimidine (1 mmol),

15   sodium acetate (14 mmol), acetic acid (86 mmol), and 4-chlorobenzene-diazonium hexafluorophosphate (1.15 mmol) in water was stirred at 25 °C for 12 h. Extraction and evaporation gave a yellow solid which was treated with zinc dust (10 mmol) and acetic acid (5.54 mmol) in EtOH-H$_2$O at 80 °C for 1 h. Extraction and chromatography gave 4-chloro-2,5-diamino-6-neopentyl-pyrimidine as a yellow solid. TLC: $R_f = 0.25$, 50 %

20   EtOAc-hexane.

Step C.   4-Chloro-2,5-diamino-6-neopentylpyrimidine was subjected to Step D, E, F in Example 2 to give 2,6-diamino-N^9-neopentyl-8-(2-(5-phosphono)furanyl)purine (22.1) as a yellow solid. mp 240 °C (decomp); Anal. calcd. for $C_{14}H_{19}N_6O_4P$ + 2.2 HBr + 0.5 acetone: C: 32.47; H: 4.25; N: 14.66. Found: C:32.31; H: 4.51; N: 14.85.

25   Similarly, 2-methylthio-N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (22.2) was also prepared from 4-amino-6-chloro-2-methylthiopyrimidine as a yellow solid. mp > 250; Anal. calcd. for $C_{15}H_{20}N_5O_4PS$ + 0.2 CH$_2$Cl$_2$ + 0.1 toluene: C: 45.08; H: 5.04; N: 16.53. Found: C:45.27; H: 5.34; N: 16.24.

81

Example 23.

Preparation of alkyloxycarbonyloxyalkyl phosphonate esters.

A solution of N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (1 mmol) in 5 mL of

anhydrous DMF is treated with N,N'-dicyclohexyl-4-morpholinecarboxamidine (5

5   mmol), and isopropyloxycarbonyloxymethyl iodide (5 mmol) which is prepared from

the commercially available chloromethyl chloroformate according to the reported

procedure, Nishimura et al. *J. Antibiotics*, 1987, *40(1)*, 81-90. The reaction mixture is

stirred for 24 h at room temperature and the solvent is removed under reduced pressure.

The resulting syrup is chromatographed on silica with 50% /50% EtOAc/Hexane to

10   yield N^9-neopentyl-8-(2-(5-diisopropyloxycarbonyloxymethyl

phosphono)furanyl)adenine.

Other alkyloxycarbonyloxymethyl, aryloxycarbonyloxymethyl , alkyl- and

arylthiocarbonyloxymethyl phosphonate esters can also be prepared following the

above described procedure.

15   Example 24.

Preparation of 1-substituted-1,3-propanediol cyclic esters of purine phosphonates.

Step A.   (*J. Org. Chem.*, 1957, 22, 589)

To a solution of 2-pyridine propanol (72.9 mmol) in acetic acid (75 mL) was added

30% hydrogen peroxide slowly. The reaction mixture was heated to 80 °C for 16 h. The

20   reaction was concentrated under vacuum and the residue was dissolved in acetic

anhydride (100 mL) and heated at 110 °C overnight. Acetic anhydride was evaporated

upon completion of reaction. Chromatography of the mixture by eluting with methanol-

methylene chloride (1:9) resulted in 10.5 g of pure 2-(1-(1,3-diacetoxy)propyl)pyridine.

Step B.   To a solution of 2-(1-(1,3-diacetoxy)propyl)pyridine (21.1 mmol) in

25   methanol-water (3:1, 40 mL) was added potassium carbonate (105.5 mmol). After

stirring for 3 h at room temperature, the reaction mixture was concentrated. The residue

was chromatographed by eluting with methanol-methylene chloride (1:9) to give 2.2 g

of crystalline 2-(1-(1,3-dihydroxy)propyl)pyridine.

Step C.   A suspension of N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (1

mmol) in 5 mL of thionyl chloride was heated at reflux temperature for 4 h. The

reaction mixture was cooled and evaporated to dryness. To the resulting residue was

added a solution of 2-(1-(1,3-dihydroxy)propyl)pyridine (1 mmol) and pyridine (2.5

5   mmol) in 3 mL of methylene chloride. After stirring at 25 °C for 4 h the reaction was

subjected to work up and chromatography to give N^9-neopentyl-8-(2-(5-(1-(2-

pyridyl)propan-1,3-yl)phosphono)furanyl)adenine (24.1) as a sticky solid. Anal. Calcd.

for $C_{22}H_{25}N_6O_4P$ + 0.75 H_2O + 1.0 HCl: C:50.97; H: 5.35; N: 16.21. Found: C:51.19,

H: 5.02; N: 15.91.

10   Following the above described procedures, other cyclic esters are also prepared,

such as N^9-neopentyl-8-(2-(5-(1-(4-pyridyl)propan-1,3-yl)phosphono)furanyl)adenine,

N^9-neopentyl-8-(2-(5-(1-(3-pyridyl)propan-1,3-yl)phosphono)furanyl)adenine, and N^9-

neopentyl-8-(2-(5-(1-phenylpropan-1,3-yl)phosphono)furanyl)adenine.

Example 25.

15   Preparation of 2-substituted-1,3-propanediol cyclic esters of purine phosphonates.

Step A.   To a solution of 2-(hydroxymethyl)-1,3-propanediol (1 g, 9.4 mmol) in

pyridine (7.5 mL) at 0 °C was added acetic anhydride (0.89 mL, 9.4 mmol) slowly. The

resulting solution was warmed to room temperature and stirred for 16 h. The reaction

was concentrated under reduced pressure and chromatographed by eluting with

20   methanol-dichloromethane (1:9) to give 510 mg of pure 2-acetoxymethyl-1,3-

propanediol.

Step B.   2-Acetoxymethyl-1,3-propanediol was coupled to N^9-neopentyl-8-(2-(5-

phosphono)furanyl)adenine following Step C of Example 24 to give N^9-neopentyl-8-(2-

(5-(2-(acetoxymethyl)propan-1,3-yl)phosphono)furanyl)adenine (25.1). mp = 164 - 165

25   °C; Anal. Calcd. for $C_{20}H_{26}N_5O_6P$: C: 51.84; H: 5.65; N: 15.11 . Found: C: 52.12; H:

5.77 ; N: 14.59.

Following the above described procedures, other cyclic esters are also prepared,

such as N^9-neopentyl-8-(2-(5-(2-(methoxycarbonyloxymethyl)-propan-1,3 -

yl)phosphono)furanyl)adenine, N^9-neopentyl-8-(2-(5-(2-(hydroxymethyl)-propan-1,3 - yl)phosphono)furanyl)adenine, N^9-neopentyl-8-(2-(5-(2,2-dihydroxymethylpropan-1,3 - yl)phosphono)furanyl)adenine. N^9-neopentyl-8-(2-(5-(2- (methoxycarbonyloxymethyl)propan-1,3 -yl)phosphono)-furanyl)adenine is prepared by

5   coupling N^9-neopentyl-8-(2-(5-phosphono)-furanyl)adenine with 2- (methoxycarbonyloxymethyl)-1,3-propanediol which was prepared as follows:

To a solution of 2-(hydroxymethyl)-1,3-propanediol (9.4 mmol) in dichloromethane (20 mL) and pyridine (7.5 mL) at 0 °C was added methyl chloroformate (9.4 mmol) slowly. The resulting solution was warmed to room

10   temperature and stirred for 16 h. The reaction was concentrated under reduced pressure and chromatographed by eluting with methanol-dichloromethane (1:4) to give 650 mg of 2-(methoxycarbonyloxymethyl)-1,3-propanediol.

Example 26.

Preparation of 8-(2-(5-hydroxyl-1,3 cyclohexyl)phosphono)furanylpurines.

15   A suspension of N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (1 mmol) in 5 mL of thionyl chloride was heated at reflux temperature for 4 h. The reaction mixture was cooled and evaporated to dryness. To the resulting residue was added a solution of cis,cis-1,3,5-cyclohexanetriol (1 mmol) and pyridine (2.5 mmol) in 3 mL of methylene chloride. After stirring at 25 °C for 24 h the reaction was subjected to work up and

20   chromatography to give N^9-neopentyl-8-(2-(5-(5-hydroxyl-1,3 cyclohexyl)phosphono)furanyl)adenine, minor isomer (26.1). mp 248 - 250 °C; Anal. Cald. for $C_{20}H_{26}N_5O_5P$ + 0.5 H_2O: C: 52.63; H: 5.96; N: 15.34. Found: C: 52.62; H: 5.70; N: 15.32; major isomer (26.2). mp 225 - 230°C; Anal. Cald. for $C_{20}H_{26}N_5O_5P$ + 0.5 H_2O: C: 52.63; H: 5.96; N: 15.34. Found: C: 52.74 ; H: 5.80; N: 15.32.

25   Following the above described procedures, N^9-phenethyl-8-(2-(5-(5-hydroxyl-1,3 cyclohexyl)phosphono)furanyl)adenine (26.3) was also prepared. Anal. Cald. for $C_{23}H_{24}N_5O_5P$ + 0.15 H_2O: C: 57.06 ; H: 5.06 ; N: 14.47. Found: C: 56.84; H: 4.83; N: 14.38.

Example 27.

Preparation of 3-(2-hydroxyethyl)phthalide.

A solution of phthalide-3-acetic acid (1 mmol) in THF was treated with borane dimethylsulfide (1.5 mmol) at 0 °C for 1h, and 25 °C for 24 h. Extraction and

5   chromatography gave 2-(3-phthalidyl)ethanol as a light yellow oil. TLC: R_f = 0.25, 50 % EtOAc - hexane.

Example 28.

Preparation of purine phosphonate amine salts

A mixture of N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine (1 mmol) and

10   tris(hydroxymethyl)aminomethane (1.05 mmol) in methanol is stirred at 25 °C for 24 h. Evaporation give N^9-neopentyl-8-(2-(5-phosphono)furanyl)adenine tris(hydroxymethyl)aminomethane salt .

Examples of the methods of the present invention includes the following. It will be understood that these examples are exemplary and that the method of the invention is

15   not limited solely to these examples.

For the purposes of clarity and brevity, chemical compounds in the following biological examples are referred to by synthetic example numbers.

Besides the following Examples, assays that may be useful for identifying compounds which inhibit gluconeogenesis include the following animal models of

20   Diabetes:

i. Animals with pancreatic b-cells destroyed by specific chemical cytotoxins such as Alloxan or Streptozotocin (e.g. the Streptozotocin-treated mouse, -rat, dog, and -monkey). Kodama, H., Fujita, M., Yamaguchi, I., *Japanese Journal of Pharmacology* 1994, *66*, 331-336 (mouse); Youn, J.H., Kim, J.K., Buchanan, T.A., *Diabetes* 1994, *43*,

25   564-571 (rat); Le Marchand, Y., Loten, E.G., Assimacopoulos-Jannet, F., et al.,

Diabetes 1978, *27*, 1182-88 (dog); and Pitkin, R.M., Reynolds, W.A., *Diabetes* 1970, *19*, 70-85 (monkey).

     ii. Mutant mice such as the C57BL/Ks db/db, C57BL/Ks ob/ob, and C57BL/6J ob/ob strains from Jackson Laboratory, Bar Harbor, and others such as Yellow Obese, T-KK, and New Zealand Obese. Coleman, D.L., Hummel, K.P., *Diabetologia* 1967, *3*, 238-248 (C57BL/Ks db/db); Coleman, D.L., *Diabetologia* 1978, *14*, 141-148 (C57BL/6J ob/ob); Wolff, G.L., Pitot, H.C., *Genetics* 1973, *73*, 109-123 (Yellow Obese); Dulin, W.E., Wyse, B.M., *Diabetologia* 1970, *6*, 317-323 (T-KK); and Bielschowsky, M., Bielschowsky, F. *Proceedings of the University of Otago Medical School* 1953, *31*, 29-31 (New Zealand Obese).

     iii. Mutant rats such as the Zucker fa/fa Rat rendered diabetic with Streptozotocin or Dexamethasone, the Zucker Diabetic Fatty Rat, and the Wistar Kyoto Fatty Rat. Stolz, K.J., Martin, R.J. *Journal of Nutrition* 1982, *112*, 997-1002 (Streptozotocin); Ogawa, A., Johnson, J.H., Ohnbeda, M., McAllister, C.T., Inman, L., Alam, T., Unger, R.H., *The Journal of Clinical Investigation* 1992, *90*, 497-504 (Dexamethasone); Clark, J.B., Palmer, C.J., Shaw, W.N., *Proceedings of the Society for Experimental Biology and Medicine* 1983, *173*, 68-75 (Zucker Diabetic Fatty Rat); and Idida, H., Shino, A., Matsuo, T., et al., *Diabetes* 1981, *30*, 1045-1050 (Wistar Kyoto Fatty Rat).

     iv. Animals with spontaneous diabetes such as the Chinese Hamster, the Guinea Pig, the New Zealand White Rabbit, and non-human primates such as the Rhesus monkey and Squirrel monkey. Gerritsen, G.C., Connel, M.A., Blanks, M.C., *Proceedings of the Nutrition Society* 1981, *40*, 237 245 (Chinese Hamster); Lang, C.M., Munger, B.L., *Diabetes* 1976, *25*, 434-443 (Guinea Pig); Conaway, H.H., Brown, C.J., Sanders, L.L. eta l., *Journal of Heredity* 1980, *71*, 179-186 (New Zealand White Rabbit); Hansen, B.C., Bodkin, M.L., *Diabetologia* 1986, *29*, 713-719 (Rhesus monkey); and Davidson, I.W., Lang, C.M., Blackwell, W.L., *Diabetes* 1967, *16*, 395-401 (Squirrel monkey).

v. Animals with nutritionally induced diabetes such as the Sand Rat, the Spiny

Mouse, the Mongolian Gerbil, and the Cohen Sucrose-Induced Diabetic Rat. Schmidt-

Nielsen, K., Hainess, H.B., Hackel, D.B., *Science* 1964, *143*, 689-690 (Sand Rat);

Gonet, A.E., Stauffacher, W., Pictet, R., et al., *Diabetologia* 1965, *1*, 162-171 (Spiny

5   Mouse); Boquist, L., *Diabetologia* 1972, *8*, 274-282 (Mongolian Gerbil); and Cohen,

A.M., Teitebaum, A., Saliternik, R., *Metabolism* 1972, *21*, 235-240 (Cohen Sucrose-

Induced Diabetic Rat).

vi. Any other animal with one of the following or a combination of the

following characteristics resulting from a genetic predisposition, genetic engineering,

10   selective breeding, or chemical or nutritional induction: impaired glucose tolerance,

insulin resistance, hyperglycemia, obesity, accelerated gluconeogenesis, increased

hepatic glucose output.

Example A: Inhibition of Human Liver FBPase

E. coli strain BL21 transformed with a human liver FBPase-encoding plasmid

15   was obtained from Dr. M. R. El-Maghrabi at the State University of New York at Stony

Brook. hlFBPase was typically purified from 10 liters of *E. coli* culture as described

(M. Gidh-Jain et al., *The Journal of Biological Chemistry* 1994, *269*, 27732-27738).

Enzymatic activity was measured spectrophotometrically in reactions that coupled the

formation of product (fructose 6-phosphate) to the reduction of

20   dimethylthiazoldiphenyltetrazolium bromide (MTT) via NADP and phenazine

methosulfate (PMS) , using phosphoglucose isomerase and glucose 6-phosphate

dehydrogenase as the coupling enzymes. Reaction mixtures (200 µL) were made up in

96-well microtitre plates, and consisted of 50 mM Tris-HCl, pH 7.4, 100 mM KCl, 5

mM EGTA, 2 mM $MgCl_2$, 0.2 mM NADP, 1 mg/mL BSA, 1 mM MTT, 0.6 mM PMS,

25   1 unit/mL phosphoglucose isomerase, 2 units/mL glucose 6-phosphate dehydrogenase,

and 0.150 mM substrate (fructose 1,6-bisphosphate). Inhibitor concentrations were

varied from 0.01 µM to 10 µM. Reactions were started by the addition of 0.002 units of

pure hlFBPase and were monitored for 7 minutes at 590 nm in a Molecular Devices
Plate Reader (37 °C).

The following Table depicts the IC_{50} values for several compounds prepared in
the Examples. AMP has an IC_{50} value of 1.0 µM in this assay.

5

Example Compound Number	IC_{50} (human liver FBPase	
2.1	5	µM
2.2	1.4	µM
2.3	3.3	µM
2.7	0.8	µM
2.10	2	µM
2.13	4.5	µM
2.14	0.9	µM
2.16	1.4	µM
4.1	100	µM
5.5	10	µM
8.1	23	µM
10.1	50	µM
11.1	0.7	µM
12.1	13	µM

Figure 2 shows the dose-dependent inhibition of hlFBPase by AMP, compound
2.7, compound 2.13, and compound 2.5.

10   *In vitro* **inhibition of rat liver and mouse liver FBPase was also determined.**

    E. coli strain BL21 transformed with a rat liver FBPase-encoding plasmid was obtained from Dr. M. R. El-Maghrabi at the State University of New York at Stony Brook, and purified as described (El-Maghrabi, M.R., and Pilkis, S.J. *Biochem. Biophys. Res. Commun.* **1991**, *176*, 137-144). Mouse liver FBPase was obtained by

5   homogenizing freshly isolated mouse liver in 100 mM Tris-HCl buffer, pH 7.4, containing 1 mM EGTA, and 10 % glycerol. The homogenate was clarified by centrifugation, and the 45-75 % ammonium sulfate fraction prepared. This fraction was redissolved in the homogenization buffer and desalted on a PD-10 gel filtration column (Biorad) eluted with same. This partially purified fraction was used for enzyme assays.

10   Both rat liver and mouse liver FBPase were assayed as described for human liver FBPase. Generally, as reflected by the higher IC_{50} values, the rat and mouse liver enzymes are less sensitive to inhibition by the compounds tested than the human liver enzyme.

    The following Table depicts the IC_{50} values for several compounds prepared in

15   the Examples:

Compound	IC_{50} Rat Liver μM	IC_{50} Mouse Liver μM
2.1	100	>20
2.2	>20	>20
2.3	>20	>20
2.7	1.25	55
2.10	>20	>20
2.13	>20	>20
2.14	>20	>20
2.16	>20	>20
4.1	>20	>20
5.5	>20	>20
8.1	>20	>20

10.1	>20	>20
11.1	>20	>20
12.1	20	>100

Example B: AMP Site Binding

To determine whether compounds bind to the allosteric AMP binding site of hlFBPase, the enzyme was incubated with radiolabeled AMP in the presence of a range of test compound concentrations. The reaction mixtures consisted of 25 mM ^{3}H-AMP

5   (54 mCi/mmol) and 0 -1000 mM test compound in 25 mM Tris-HCl, pH 7.4, 100 mM KCl and 1 mM $MgCl_2$. 1.45 mg of homogeneous FBPase ($\pm$1 nmole) was added last. After a 1 minute incubation, AMP bound to FBPase was separated from unbound AMP by means of a centrifugal ultrafiltration unit ("Ultrafree-MC", Millipore) used according to the instructions of the manufacturer. The radioactivity in aliquots (100 μl)

10   of the upper compartment of the unit (the retentate, which contains enzyme and label) and the lower compartment (the filtrate, which contains unbound label) were quantified using a Beckman liquid scintillation counter. The amount of AMP bound to the enzyme was estimated by comparing the counts in the filtrate (the unbound label) to the total counts in the retentate.

15   As evident from Fig. 3, both aminoimidazolecarboxamideriboside monophosphate (ZMP) and compound 2.2 displaced AMP from hlFBPase in a dose-dependent manner, indicating that they bind to the same site on the enzyme as AMP. As expected, compound 2.2, a more potent hlFBPase inhibitor than ZMP (IC_{50}'s = 5 and 12 μM, respectively), had a lower ED_{50} for AMP displacement than ZMP (35 vs

20   250 μM).

Example C: AMP Site/Enzyme Selectivity

To determine the selectivity of compounds towards FBPase, effects of FBPase inhibitors on 5 key AMP binding enzymes were measured using the assays described below:

Adenosine Kinase: Human adenosine kinase was purified from an *E. coli* expression

system as described by Spychala et al. (Spychala, J., Datta, N.S., Takabayashi, K.,

Datta, M., Fox, I.H., Gribbin, T., and Mitchell, B.S. *Proc. Natl. Acad. Sci. USA* 1996,

93, 1232-1237). Activity was measured essentially as described by Yamada et al.

5   (Yamada, Y., Goto, H., Ogasawara, N. *Biochim. Biophys. Acta* 1988, *660*, 36-43.) with

a few minor modifications. Assay mixtures contained 50 mM TRIS-maleate buffer, pH

7.0, 0.1 % BSA, 1 mM ATP 1 mM MgCl$_2$, 1.0 µM [U-^{14}C] adenosine (400-600

mCi/mmol) and varying duplicate concentrations of inhibitor. ^{14}C-AMP was separated

from unreacted ^{14}C-adenosine by absorption to anion exchange paper (Whatman) and

10   quantified by scintillation counting.

Adenosine Monophosphate Deaminase: Porcine heart AMPDA was purified essentially

as described by Smiley *et al.* (Smiley, K.L., Jr, Berry, A.J., and Suelter, C.H. *J. Biol.*

Chem. 1967, *242*, 2502-2506) through the phosphocellulose step. Inhibition of

AMPDA activity was determined at 37 °C in a 0.1 mL assay mixture containing

15   inhibitor, ~0.005U AMPDA, 0.1 % bovine serum albumin, 10 mM ATP, 250 mM KCl,

and 50 mM MOPS at pH 6.5. The concentration of the substrate AMP was varied from

0.125 - 10.0 mM. Catalysis was initiated by the addition of enzyme to the otherwise

complete reaction mixture, and terminated after 5 minutes by injection into an HPLC

system. Activities were determined from the amount of IMP formed during 5 minutes.

20   IMP was separated from AMP by HPLC using a Beckman Ultrasil-SAX anion

exchange column (4.6 mm x 25 cm) with an isocratic buffer system (12.5 mM

potassium phosphate, 30 mM KCl, pH 3.5) and detected spectrophotometrically by

absorbance at 254 nm.

Phosphofructokinase: Enzyme (rabbit liver) was purchased from Sigma. Activity was

25   measured at 30 °C in reactions in which the formation of fructose 1,6-bisphosphate was

coupled to the oxidation of NADH via the action of aldolase, triosephosphate

isomerase, and α-glycerophosphate dehydrogenase. Reaction mixtures (200 µl) were

made up in 96-well microtitre plates and were read at 340 nm in a Molecular Devices
Microplate Reader. The mixtures consisted of 200 mM Tris-HCl pH 7.0, 2 mM DTT, 2
mM MgCl2, 0.2 mM NADH, 0.2 mM ATP, 0.5 mM Fructose 6-phosphate, 1 unit
aldolase/mL, 3 units/mL triosephosphate isomerase, and 4 units/mL

5   α-glycerophosphate dehydrogenase. Test compound concentrations ranged from 1 to
500 µM. Reactions were started by the addition of 0.0025 units of phosphofructokinase
and were monitored for 15 minutes.

Glycogen Phosphorylase: Enzyme (rabbit muscle) was purchased from Sigma.
Activity was measured at 37 °C in reactions in which the formation of glucose 1-

10   phosphate was coupled to the reduction of NADP via phosphoglucomutase and glucose
6-phosphate dehydrogenase. Assays were performed on 96-well microtitre plates and
were read at 340 nm on a Molecular Devices Microplate Reader. Reaction mixtures
consisted of 20 mM imidazole, pH 7.4, 20 mM MgCl$_2$, 150 mM potassium acetate, 5
mM potassium phosphate, 1 mM DTT, 1 mg/mL BSA, 0.1 mM NADP, 1 unit/mL

15   phosphoglucomutase, 1 unit/mL glucose 6-phosphate dehydrogenase, 0.5 % glycogen.
Test compound concentrations ranged from 1 to 500 µM. Reactions were started by the
addition of 17 µg enzyme and were monitored for 20 minutes.

Adenylate Kinase: Enzyme (rabbit muscle) was purchased from Sigma. Activity was
measured at 37 °C in reaction mixtures (100 µl) containing 100 mM Hepes, pH 7.4, 45

20   mM MgCl$_2$, 1 mM EGTA, 100 mM KCl, 2 mg/mL BSA, 1 mM AMP and 2 mM ATP.
Reactions were started by addition of 4.4 ng enzyme and terminated after 5 minutes by
addition of 17 µl perchloric acid. Precipitated protein was removed by centrifugation
and the supernatant neutralized by addition of 33 µl 3 M KOH/3 M KH$_2$CO$_3$. The
neutralized solution was clarified by centrifugation and filtration and analyzed for ADP

25   content (enzyme activity) by HPLC using a YMC ODS AQ column (25 X 4.6 cm). A
gradient was run from 0.1 M KH$_2$PO$_4$, pH 6, 8 mM tetrabutyl ammonium hydrogen
sulfate to 75 % acetonitrile. Absorbance was monitored at 254 nM.

Compound 2.1, a 5 µM hIFBPase inhibitor, was essentially inactive in all of the above described assays except for the AMP deaminase screen: half-maximal inhibition of AMP deaminase was observed at almost the same concentration as the IC_{50} for FBPase. Compound 2.7 (hIFBPase IC_{50} = 0.8 µM), in addition to being essentially

5 without effect on adenosine kinase, adenylate kinase, glycogen phosphorylase, and phosphofructokinase, was only a weak inhibitor of AMP deaminase (IC_{50} = 390 µM). The data suggest that compound 2.7 binds to hIFBPase in a highly selective manner. The following Table gives the selectivity data for compounds 2.1 and 2.7.

SELECTIVITY

	Compound 2.1	Compound 2.7
FBPase (inh.)	5.0 µM	0.8 µM
Adenosine Kinase (inh.)	>>10	>>100
Adenylate Kinase (inh.)	>>500	>>500
AMP Deaminase (inh.)	6.7	390
Glycogen Phosphorylase (act.)	>>250	>>100
Phosphofructokinase (act.)	>>200	>>100

10

Example D: Inhibition of Gluconeogenesis in Rat Hepatocytes

Hepatocytes were prepared from overnight fasted Sprague-Dawley rats (250-300 g) according to the procedure of Berry and Friend (Berry, M.N., Friend, D.S., *J. Cell. Biol.* 1969, *43*, 506-520) as modified by Groen (Groen, A.K., Sips, H.J., Vervoom,

15 R.C., Tager, J.M., *Eur. J. Biochem.* 1982, *122*, 87-93). Hepatocytes (75 mg wet weight/mL) were incubated in 1 mL Krebs-bicarbonate buffer containing 10 mM Lactate, 1 mM pyruvate, 1 mg/mL BSA, and test compound concentrations from 1 to 500 µM. Incubations were carried out in a 95 % oxygen, 5 % carbon dioxide atmosphere in closed, 50-mL Falcon tubes submerged in a rapidly shaking water bath

20 (37 °C). After 1 hour, an aliquot (0.25 mL) was removed, transferred to an Eppendorf

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tube and centrifuged. 50 µl of supernatant was then assayed for glucose content using a
Sigma Glucose Oxidase kit as per the manufacturer's instructions.

Compound 2.1 and compound 2.7 inhibited glucose production from
lactate/pyruvate in isolated rat hepatocytes in a dose-dependent manner, with IC_{50}'s of

5   90 and 4.5 µM, respectively (Figure 5). IC_{50}'s for other select compounds in this assay
are shown in the Table below:

Compound	IC_{50} Glucose Production, µM
2.2	90
2.6	18
2.10	24
2.13	50
2.14	7.5
2.16	12
16.4	3

FPBase from rat liver is less sensitive to AMP than that from human liver. IC_{50}
values are correspondingly higher in rat hepatocytes than would be expected in human
hepatocytes.

10 **Example E: Effect of Compound 2.7 on gluconeogenesis from Dihydroxyacetone
in Rat Hepatocytes: Glucose Production Inhibition and Fructose 1,6-bisphosphate
Accumulation**

Isolated rat hepatocytes were prepared as described in Example D and incubated
under the identical conditions described except that lactate/pyruvate was replaced by 10

15 mM dihydroxyacetone, a substrate which feeds into the gluconeogenic pathway at a
step just prior to FBPase. Reactions were terminated by removing an aliquot (250 µL)
of cell suspension and spinning it through a layer of oil (0.8 mL silicone/mineral oil,
4/1) into a 10 % perchloric acid layer (100 µL). After removal of the oil layer, the

acidic cell extract layer was neutralized by addition of 1/3rd volume of 3 M KOH/3 M

KH$_2$CO$_3$. After thorough mixing and centrifugation, the supernatant was analyzed for

glucose content as described in Example D, and also for fructose-1,6-bisphosphate.

Fructose-1,6-bisphosphate was assayed spectrophotometrically by coupling its

5   enzymatic conversion to glycerol 3-phosphate to the oxidation of NADH, which was

monitored at 340 nm. Reaction mixtures (1 mL) consisted of 200 mM Tris-HCl, pH

7.4, 0.3 mM NADH, 2 units/mL glycerol 3-phsophate dehydrogenase, 2 units/mL

triosephosphate isomerase, and 50-100 μl cell extract. After a 30 minute preincubation

at 37 °C, 1 unit/mL of aldolase was added and the change in absorbance measured until

10   a stable value was obtained. 2 moles of NADH are oxidized in this reaction per mole of

fructose-1,6-bisphosphate present in the cell extract.

        As shown in Figure 4A, compound 2.7 inhibited glucose production from

dihydroxyacetone in rat hepatocytes (IC$_{50}$ approx. 5 μM) as effectively as from lactate

pyruvate (IC$_{50}$ 4.5 μM, Figure 5). This data confirms that the site of action of the

15   compound is in the last four steps of the gluconeogenic pathway. The dose-dependent

accumulation of fructose-1,6-bisphosphate (the substrate of FBPase) that occurs upon

cell exposure to compound 2.7 (Figure 4B) is consistent with the inhibition of FBPase,

the second to last enzyme in the pathway.

Example F: Blood Glucose Lowering in Fasted Rats

20   Sprague Dawley rats (250-300 g) were fasted for 18 hours and then dosed

intraperitoneally either with saline or with 35, 45, and 60 mg/kg compound 16.4, a

prodrug of compound 2.7. The vehicle used for drug administration was

dimethylsulfoxide. Blood samples were obtained from the tail vein of conscious

animals just prior to injection and then at half-hourly intervals. Blood glucose was

25   measured using a HemoCue Inc. glucose analyzer according to the instructions of the

manufacturer.

Figure 6 shows the profound glucose lowering elicited by treatment with compound 16.4. The duration of action was dose-dependent and ranged from 2 to 6 hours.

Example G: Analysis of Drug Levels and Liver Fructose-1,6-bisphosphate
5   **Accumulation In Rats**

Sprague-Dawley rats (250-300 g) were fasted for 18 hours and then dosed intraperitoneally either with saline (n = 3) or 20 mgs/kg compound 2.7 (n = 4). The vehicle used for drug administration was 10 mM bicarbonate. One hour post injection rats were anesthetized with halothane and a liver biopsy (approx. 1 g) was taken as well
10   as a blood sample (2 mL) from the posterior vena cava. A heparin flushed syringe and needle were used for blood collection. The liver sample was immediately homogenized in ice-cold 10% perchloric acid (3 mL), centrifuged, and the supernatant neutralized with 1/3rd volume of 3 M KOH/3 M KH_2CO_3. Following centrifugation and filtration, 50 µL of the neutralized extract was analyzed for compound 2.7 content by HPLC. A
15   reverse phase YMC ODS AQ column (250 X 4.6 cm) was used and eluted with a gradient from 10 mM sodium phosphate pH 5.5 to 75 % acetonitrile. Absorbance was monitored at 310 nm. The concentration of fructose-1,6-bisphosphate in liver was also quantified using the method described in Example E. Blood glucose was measured in the blood sample as described in Example F. Plasma was then quickly prepared by
20   centrifugation and extracted by addition of methanol to 60 % (v/v). The methanolic extract was clarified by centrifugation and filtration and then analyzed by HPLC as described above.

Compound 2.7 lowered blood glucose from 82 ± 3 to 28 ± 9.9 mg/dL within one hour (Figure 7). Drug levels measured in plasma and liver were 38.5 ± 7 µM and 51.3
25   ± 10 nmoles/ g, respectively. As shown in Figure 8, a 10-fold elevation of fructose-1,6-bisphosphate levels was found in the livers from the drug-treated group, consistent with the inhibition of glucose production at the level of FBPase in the gluconeogenic pathway.

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Example H: Blood Glucose Lowering in Zucker Diabetic Fatty Rats

Zucker Diabetic Fatty rats purchased at 7 weeks of age were used at age 16 weeks in the 24-hour fasted state. The rats were purchased from Genetics Models Inc. and fed the recommended Purina 5008 diet (6.5 % fat). Their fasting hyperglycemia at 24 hours ranged from 150 mg/dL to 310 mg/dL blood glucose.

Compound 2.7 was administered at a dose of 50 mg/kg by intraperitoneal injection (n = 6). The stock solution was made up at 25 mg/mL in deionized water and adjusted to neutratility by dropwise addition of 5 N NaOH. 5 control animals were dosed with saline. Blood glucose was measured at the time of dosing and 2 hours post dose as described in Example F.

As shown in Figures 9A and 9B, blood glucose was lowered in the drug-treated group by an average of almost 20 % (p < 0.0001 relative to the control animals).

Example I: Inhibition of Gluconeogenesis in Zucker Diabetic Fatty Rats

Three 20-week old Zucker Diabetic Fatty rats were dosed with compound 2.7 and three with saline as described in Example H. Fifteen minutes post-injection, the animals were anesthetized with sodium pentobarbitol (30 mgs, i.p.) and ^{14}C-bicarbonate (20 μCi /100 g of body weight) was administered via the tail vein. Blood samples (0.6 mL) were obtained by cardiac puncture 10 and 20 minutes post tracer injection. Blood (0.5 mL) was diluted into 6 mL deionized water and protein precipitated by addition of 1 mL zinc sulfate (0.3 N) and 1 mL barium hydroxide (0.3 N). The mixture was centrifuged (20 minutes, 1000X g) and 5 mL of the resulting supernatant was then combined with 1 g of a mixed bed ion exchange resin (1 part AG 50W-X8, 100-200 mesh, hydrogen form and 2 parts of AG 1-X8, 100-200 mesh, acetate form) to separate ^{14}C-bicarbonate from ^{14}C-glucose. The slurry was shaken at room temperature for four hours and then allowed to settle. An aliquot of the supernatant (0.5 mL) was then counted in 5 mL scintillation cocktail.

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As shown in Figure 10, compound 2.7 reduced the incorporation of ^{14}C-bicarbonate into glucose by 75 %; therefore gluconeogenesis was clearly inhibited by the drug.

Example J: Blood Glucose Lowering in Streptozotocin-treated Rats

5   Diabetes is induced in male Sprague-Dawley rats (250-300g) by intraperitoneal injection of 55 mg/kg streptozotocin (Sigma Chemical Co.). Six days later, 24 animals are selected with fed blood glucose values (8 am) between 350 and 600 mg/dL and divided into two statistically equivalent groups. Blood glucose is measured in blood obtained from a tail vein nick by means of a HemoCue Inc. (Mission Viejo, CA)

10   glucose analyzer. One group of 12 will subsequently receive inhibitor (100 mg/kg intraperitoneally) and the other 12 ("controls") an equivalent volume of saline. Food is removed from the animals. Blood glucose is measured in each animal four hours after dosing, and a second dose of drug or saline is then administered. Four hours later, a final blood glucose measurement is made.

15   **Example K: Evaluation of Compound 16.4 as a Prodrug in Rat Hepatocytes -**
Intracellular Delivery of Compound 2.7

        Rat hepatocytes were prepared and incubated as in Example D, except that the test compound, 16.4, was added to yield a final concentration of 10 μM. Aliquots of the cell suspension were taken at 0, 5, 10, 20, 30, 45, and 60 minutes after drug exposure.

20   The cells were extracted and analyzed for compound 2.7 content by HPLC as described in Example L. Absorbance of the HPLC column eluate was monitored at 310 nm. Quantitation of intracellular compound 2.7 was done by comparison to authentic standards of known concentration. As shown in Figure 11A, compound 16.4 rapidly delivered high levels of compound 2.7 into the hepatocytes; a concentration of

25   approximately 80 nmoles/g was achieved within 10 minutes. These data indicate that compound 16.4 readily penetrates cells and is efficiently de-esterified to the parent

compound, 2.7, intracellularly. Furthermore, as shown in Figure 11B, compound 16.4 inhibited glucose production in rat hepatocytes.

Example L: Estimation of the oral bioavailability of prodrugs of phosphonic acids:

5   Prodrugs were dissolved in 10 % ethanol/90 % polyethylene glycol (mw 400) and administered by oral gavage at doses of approximately 20 or 40 mg/kg parent compound equivalents to 6-hour fasted, Sprague Dawley rats (220-240 g). The rats were subsequently placed in metabolic cages and urine was collected for 24 hours. The quantity of parent compound excreted into urine was determined by HPLC analysis. An

10   ODS column eluted with a gradient from potassium phosphate buffer, pH 5.5 to acetonitrile was employed for these measurements. Detection was at 310-325 nm. The percentage oral bioavailability was estimated by comparison of the recovery in urine of the parent compound generated from the prodrug, to that recovered in urine 24 hours after intravenous administration of unsubstituted parent compound at approximately 10

15   mg/kg. Parent compounds were typically dissolved in dimethyl sulfoxide, and administered via the tail vein in animals that were briefly anesthetized with halothane.

For compound **16.4**, a prodrug of compound **2.7**, 6.2 % of an oral dose of approximately 20 mg/kg was recovered in urine. For compound **2.7**, 76.8 % of an intravenous dose of approximately 10 mg/kg was recovered. The oral bioavailability of

20   compound **16.4** was therefore calculated to be 6.2/76.8, or approximately 8 %. The oral bioavailability of compound **16.5** was also estimated following the above described protocol to be 5.3 %.

Example M. Glucose lowering following oral administration of FBPase inhibitors:

FBPase inhibitor was administered by oral gavage at doses of 30, 100 and 250 mg/kg to

25   18-hour fasted, Sprague Dawley rats (250-300g; n= 4-5/group). The compound was prepared in deionized water, adjusted to neutrality with sodium hydroxide, and brought

into solution by sonication prior to administration. Blood glucose was measured

immediately prior to dosing, and at 1 hour intervals thereafter. Blood samples were

obtained from the tail vein, and measurments made by means of a Hemocue glucose

analyzer (Hemocue Inc, Mission Viejo, California) used according to the

5   manufacturer's instructions.